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GEOGRAPHIC VARIATION IN SKIN PIGMENTATION AND DERMAL GLANDS IN THE NORTHERN LEOPARD FROG, RANA PIPIENS

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Abstract

I assessed 31 variables (18 factor scores from 31 multistate characters, logarithms of 6 linear and of the square roots of 6 area measurements, and specimen size) and composite variables based on *a priori* combinations of characters on dried skins of 828 *Rana pipiens* from Canada and the NE USA. I conclude that: (1) The first pattern of pigmentation variation is the greater extent of dark spotting in warmer, moister climates (=Gloger's Rule), which may result from crypsis by background-matching or the more aquatic niche of less spotted *Rana pipiens* which are allopatric from aquatic species of *Rana*. (2) Variation in the extent of dermal secretory glands is largely independent of variation in pigmentation. Glands are more extensive where water is more widespread and in the north; the glands may protect the frogs from some stress associated with submergence or wintering in water (perhaps infection or osmotic losses). (3) Field, lake edge, and marsh habitats are associated with linear pattern elements and reduced leg spotting. This may conceal frogs against grassy backgrounds. (4) These 3 kinds of variation emerge in factor analysis of multistate data, DFs among localities, and CCs with environmental and geographic variables. The first two remain uncorrelated and show the same patterns of geographic variation in data from 864 other specimens. (5) Much variation in the number of spots is within rather than among populations, and among-populations variation in dorsal spot number is inverse to that in spot area. (6) A marked Kluge-Kerfoot effect is evident. (7) The sexes differ in pigmentation, but not in dermal glands. Males are less patterned on the head and back than females, but are more patterned on lateral and ventral surfaces. Males spend more of the spring breeding period in aquatic habitat than females, and the male coloration is more like that of aquatic frogs. (8) Apparent 'burnsi' (dorsally unspotted frogs) occur at scattered localities in the east, and apparent 'kandiyohi' (frogs with dark interspot reticulations) occur at Long Point, Ontario.

Some methodological innovations are: (1) The use of TSA as a 'null hypothesis' of difference with distance to which hypothesized environmental or taxonomic relationships are compared. (2) The use of CC analysis between specimen and geographic variables (terms of TSA) to extract

Résumé

Rana pipiens a été collectée au Canada et au nord-est des États-Unis de 1971 à 1974. 828 peaux ont été préservées par séchage et pressage, et 46 critères ont été transformés en 31 variables (18 valeurs factorielles à partir de 31 critères à états multiples, logarithmes de 6 mesures linéaires, et racines carrées de 6 mesures de surface, et de dimensions des spécimens) et variables composées basées sur des idées *à priori* de types de variations qui pourraient se produire.

On conclut que: (1) Le premier mode de variation de pigmentation est une plus grande extension de tachetures foncées dans les climats plus chauds et plus humides (= règle de Gloger), ce qui pourrait être le résultat d'un crypsisme par camouflage avec l'arrière-fond, ou d'une niche plus aquatique des *Rana pipiens* moins tachetées qui sont allopatriques aux espèces aquatiques de *Rana*. (2) La variation dans le nombre de glandes sécrétrices dermales est indépendante de la variation dans la pigmentation. Les glandes sont plus nombreuses où l'eau est plus fréquente, et au nord ; ces glandes pourraient protéger les grenouilles du stress (soit infection ou pertes osmotiques) associé avec la submergence ou l'hivernage dans l'eau. Vers le nord, une croissance dans le nombre de glandes dermales pourrait être une règle générale chez les *Rana* semi-terrestrielles. (3) Il y a une association entre champs, bords de lacs et habitats marécageux, et les éléments de patrons linéaires et la réduction dans la tacheture de la patte. Ceci pourrait camoufler les grenouilles dans des arrière-plans herbeux. (4) Ces trois types de variations émergent dans l'analyse factorielle de données à états multiples, de fonctions discriminantes dans les localités, et de corrélations canoniques avec les variables environnementales et géographiques. Les deux premiers types demeurent sans rapport et montrent les mêmes modes de variation géographique dans des données de 864 autres spécimens. (5) La majorité de variation dans le nombre de taches est à l'intérieur de chaque population plutôt qu'entre populations, et la variation dans le nombre de taches dorsales est inverse à la variation de surface de chaque tache. (6) Un effet Kluge-Kerfoot marqué (correspondant à une variabilité à l'intérieur des populations et entre populations) est évident. (7) La pigmentation diffère entre les

uncorrelated geographic patterns from the specimen data. (3) The use of habitat and relief descriptors from topographic maps and of high-order TSAs of continental climate variation.

sexes, mais non les glandes dermales. Les mâles ont un motif plus discret que les femelles sur la tête et le dos, mais plus évident sur les surfaces latérales et ventrales. Les mâles consacrent plus de temps dans les habitats aquatiques que les femelles, pendant la période de reproduction printanière, et la coloration des mâles est plus comparable à celle des grenouilles aquatiques. (8) Les *Rana pipiens* de 'burnsi' apparents (grenouilles sans taches) apparaissent dans des localités éparses dans l'est, et les 'kandiyohi' apparents se manifestent à Long Point, en Ontario.

Certaines innovations méthodologiques sont: (1) L'emploi des analyses de surface comme 'hypothèses nulle' pour comparer la différence entre la distance et des relations environnementales ou taxonomiques hypothétiques. (2) L'utilisation de l'analyse de corrélations canoniques entre les variables de spécimens et les variables géographiques (termes d'analyse de surface) pour en extraire des patrons géographiques sans corrélation avec les données des spécimens. (3) L'emploi de descripteurs d'habitat et de relief à partir de cartes topographiques, et l'emploi d'analyses de surface d'ordre élevé des variations de climat continental.

[Traduit par Michèle Steigerwald]

Epigraph

This is, essentially, why we cannot any longer believe in the scholastic maxim *Simplex sigillum veri* ('simplicity is the seal of truth'), because we know that all of our constructions are defective, hence corrigible, since, deliberately or not, they involve the neglect of an unknown number of factors. Factual theories apply exactly to schematic, impoverished models or images, and only inexactly to the real referents of these pictures. *The simpler the theoretical model the coarser or more unrealistic it will be.* We need not wait for empirical tests to make sure that *all* our factual theories are, strictly speaking, false — or, put in an optimistic way, only partially true. We know this beforehand if only because *we have introduced falsities into them, in the form of simplifications*, as shown by historical experience and by an analysis of the way factual theories are built. Conceptual economy is therefore a sign and a test of transitoriness, *i.e.*, of partial falsity — to be superseded by a lesser falsity. *Simplex sigillum falsi.*

S. Bunge (1963) p. 112-113.

Part I: General Introduction

The organization of this work follows only roughly the conventions for reporting experimental science, as I have presented the results of each analysis directly after the description of the methods employed. Part I is introductory, Part II deals mainly with methodology and materials, Parts III and IV present the results of most of the analyses, Part V is largely a discussion of the adaptive interpretation of the findings, and Part VI is an abstract of the conclusions, but 'methods', 'results', and 'discussion' are found throughout the text.

This study is exploratory, descriptive, and qualitative, so I have not felt that tests of statistical significance are appropriate in many instances, but all of the results that I mention in the text are formally endorsed at least at the $p<0.05$ level of significance. I have approached the analysis of the data cautiously and have tried to interpret only those patterns of variation which emerge repeatedly in different analyses or which bear on hypotheses previously suggested in the literature. This caution is dictated by the difficulties of attributing adaptive causes to correlations in data which are remote from the selective forces supposed to be responsible for the variation (Fretwell, 1972).

The attributes used here are those which can be conveniently assessed on dried skins: pigmentation and dermal gland characters. I will discuss morphometric variation among these samples elsewhere in terms of skeletal measurements.

I have set out to (1) find sets of characters which vary independently among populations, (2) relate variation in these to variation within populations, (3) see to what extent the variation among populations can be described by geographic patterns, and (4) relate the variation among populations to variation in climatic and topographic factors.

I attempt geographic tests of (1) Schaaf and Smith's (1970) hypothesis that dark dorsal coloration in frogs serves an energy-coloration function and increases in colder climates, and of the hypothesis that dermal mucous glands, which provide water for evaporative cooling, should increase in size or extent in thermally stressful habitats (Lillywhite & Licht, 1975; Schmid, 1965).

Finally, I test the generality of the conclusions drawn from the skin specimens by attempting to replicate them using fluid-preserved specimens from many more localities.

Abbreviations are listed in Table 1.

Table 1. Abbreviations.

Analyses & Resulting Variables	
ANOVA	Analysis of Variance
PC, PCA	Principal Component, PC Analysis
CC, CCA	Canonical Correlation, CC Analysis
DF, DFA	Discriminant Function, DFs Analysis
TS, TSA	Trend Surface, TS Analysis
WSI	Weighted Separation Index
Programmes	
SPSS	Statistical Package for the Social Sciences (Nie *et al.*, 1976)
NT-SYS	Numerical Taxonomy System of Multivariate Statistical Programs (Rohlf *et al.*, 1974)
Statistical Terms	
n	Number of observations
s.d.	Standard Deviation
r	Product-moment correlation coefficient
'*' denotes multiplication; '**' denotes exponentiation	
Location of specimens	
AMNH	American Museum of Natural History, New York
CU	Cornell University, Ithaca, New York
MCZ	Museum of Comparative Zoology, Harvard University
NMNS	National Museum of Natural Sciences, National Museums of Canada, Ottawa
ROM	Royal Ontario Museum, Toronto
FWS	My field numbers, specimens deposited in NMNS but not yet catalogued there.

Leopard Frogs

Leopard frogs are medium- to large-sized, semiterrestrial, North and Central American *Rana*, characterized by conspicuous dark dorsal spots. Until recently they were considered to be one species (*R. pipiens*; Moore, 1944), but the discovery of call differences among populations has led to the division of *R. pipiens* into at least seven species in the United States and others in Mexico and Central America (Pace, 1974; Platz & Mecham, 1979). The name *Rana pipiens* is retained by the leopard frogs of Canada and the northern and montane United States. Pace (1974)

applied the old name *R. utricularia* to the leopard frogs of the southeastern United States, and the hitherto unnamed leopard frogs of the southern Great Plains were described as *R. blairi* by Mecham *et al.* (1973; see Figure 13). Since *R. palustris*, the Pickerel Frog, seems to be no more distantly related to the leopard frogs than the leopard frogs are to each other (Moore, 1946; Pace 1974) I refer to all of these species as the *Rana pipiens* complex.

This study describes variation within the restricted *Rana pipiens* through much of its range outside of the Cordillera of the United States. I know of no evidence of species-level differences among populations of leopard frogs in Canada: the vocalizations and diagnostic character states of *R. pipiens* are uniform in these populations.

Rana pipiens overwinter in still or flowing water, rarely on land (Emery *et al.*, 1972). In the spring, at water temperatures of 9-12° C, they move into shallow water or small ponds to breed. The males precede the females to the breeding sites, gather in aggregations, and call from the surface of the water. The females move into the aggregations of males, amplex, and attach masses of eggs to submerged vegetation (Pace, 1974). The eggs hatch into tadpoles which metamorphose in about 60-80 days (Wright, 1914). The frogs characteristically spend the summer in marsh or meadowland; males and yearlings are found in wetter habitat than the larger adult females (Dole, 1965). *R. pipiens* can obtain water from moist soil and dew, and need not visit open water during the summer (Tracy, 1976; Dole, 1967b). The home range is generally small, but the frogs make extensive movements on rainy nights (Dole, 1967a) and recently-metamorphosed frogs may move as far as 5 km from the site of metamorphosis (Dole 1971). Sexual maturity may be reached in one to three years (Pace, 1974), depending on growing conditions.

Functions of Pigmentation for Frogs

The individual variability and slight sexual dichromatism of *R. pipiens* probably exclude communication as a function of much, if any, of its colour pattern. *Rana* are much preyed upon by birds and other visual predators, so crypsis (*e.g.* Norris & Lowe, 1964) will be more important than energy-coloration (colour patterns that optimize the reflection or absorption of solar radiation; Hamilton, 1973) in their coloration. The considerable colour changes frogs undergo further mask energy-coloration, effects of pattern differences (Hogben & Kirk, 1944), but such changes may themselves may be thermal adaptations, and variation in colour within limits imposed by crypsis may improve, if not optimize, the absorption or reflection of light (Schwalm *et al.*, 1977). The question of whether dark animals are cryptic, thermal opportunists, or both, is complicated by the association between mesic habitats and dark soil colour (due to the accumulation of organic matter, shade from taller vegetation, and the direct darkening effect of water); this is a complex problem (Jones *et al.*, 1977; Papageorgis, 1975), which will not be resolved by the present study.

Rana may thermoregulate closely by basking (Lillywhite, 1970, 1971a), but Tracy (1975, 1976) doubted that behaviour or colour differences are of much thermal importance to *Rana*, because variation in radiative heat gain is so well buffered by evaporative cooling. This assumes that frogs have access to water-saturated soil (Tracy, 1975), which may not be the case when thermal selection for reduced dark pigmentation is taking place (Dole, 1967a&b). Leopard frogs active during the summer have body temperatures of 18-32° C, and "it is obvious from the behavior of these frogs that they are strongly heliothermic" (Brattstrom, 1963, p. 249), but the bearing of their coloration on their heat gain is unknown for the present.

The dominant function of pigmentation in *Rana pipiens* is doubtless disruptive concealment, an hypothesis suggested by the agreement between the disposition of the pigmentation and the principles of disruptive coloration (Cott, 1940). Visual disruption is effected by highly contrasting elements on a background-matching ground colour, a condition met in leopard frogs by the dark spotting on the green or brown dorsal surface, which is countershaded to the white venter. The disruptive elements should have a maximum contrast with the ground colour and make the image of the animal interpretable as more than one object; in *Rana pipiens* melanophores are so arranged that the greatest and least concentrations of melanin are adjacent at the edges of the spots (Smith-Gill, 1973; Cott, 1940, figures 9 & 11). Not only are the spots individually more prominent visual elements than the outline of a motionless frog, but the light dorsolateral folds trisect the field of dark spots. There is coincident disruptive coloration in the spots of the hind legs, which often are adjacent from one

segment of the folded leg to another (Cott, 1940, figure 21; Wright & Wright, 1949, plates CI, figure 5, CII, lowest figure). The eye is concealed by the canthus-rostralis pigmentation and spots above the tympanum (Cott, 1940, figure 31; Smith, 1961, figure 89), and there is, perhaps, background picturing of grass and shadow by the dorsolateral folds, the jawlines, and other linear pattern elements (Cott, 1940, plate 24(1)).

Background picturing of grassy habitats by striped patterns provides protection from visual predation (Turner, 1961), and Stewart (1974), Pyburn (1961), Nevo (1973a), Fishbeck and Underhill (1971), and Schueler & Cook (1980) have described variation among populations of frogs that they interpreted as greater frequencies of striping in grassier habitats.

Discussion of light-mediated adaptive functions of leopard frog pigmentation is speculative, but Merrell and Rodell (1968) found that burnsi (unspotted) *Rana pipiens* were selectively favoured over spotted individuals while wintering in lakes in Minnesota. Burnsi occurred more frequently among living than among dead frogs found in the early spring, and more frequently in spring samples than in samples taken the previous fall. They suggested that "since a sizeable winter kill of fishes was typically observed in the lakes where dead frogs were found. . . a major factor in the death of both frogs and fish was probably depletion of the oxygen supply" and that "the *burnsi* type may be better able to tolerate low oxygen tensions than *pipiens*" (*ibid.*, p. 286). Exposure of tadpoles to low oxygen tensions or high temperatures contributes to the full phenotypic expression (spotlessness) of the burnsi gene at metamorphosis (Davison, 1964). Both of these lines of evidence point to some relationship between the burnsi allele and oxygen-poor environments. Merrell (1972) has also found that in the laboratory tadpoles with the other well-known pigmentation polymorph allele, kandiyohi, metamorphose sooner than their wild-type siblings.

Possible Functions of Ranid Dermal Glands

The structures that I call 'dermal glands' are serous (or granular, or poison) glands and enlarged mucous glands similar to those that comprise the dorsolateral folds. Mucous glands, which release an acidic secretion containing sulfate, carboxylic acids, and carbohydrate (Dapson, 1970) in synchronous pulses (Lillywhite, 1971b) are found all over the frog. Serous glands, which release a secretion of sulphur-containing amino acids, carboxylic acid, tyrosine, catecholamines, phospholipoprotein, and carbohydrate (Dapson *et al.*, 1973) in response to catecholamines, which may originate either adrenally or from sympathetic neurons (Benson & Hadley, 1969), are found mostly in the dorsolateral folds (Bovbjerg, 1963) and (by inference) in the similar ridges of enlarged glands elsewhere on the dorsal surface of the skin. The serous glands are homologous to glands which produce various toxic secretions in other anurans (Noble & Noble, 1944; Neuwirth *et al.*, 1979). *Rana pipiens* is not usually thought of as toxic, though *R. palustris*, which has, both dorsally and ventrally, large areas of serous glands (pers. obs.), is rejected as food by snakes (Cott, 1940). Welsh & Zipf (1966) found much more 5-hydroxytriptamine in the serous glands of 'semiterrestrial' *Rana* (*R. pipiens, utricularia, aurora, & sylvatica*) than in those of aquatic species (*R. catesbeiana, clamitans, & grylio*). They suggested, perhaps implausibly, that this substance is uniquely deterrent to terrestrial predators.

One function of the mucous glands is evaporative thermoregulation: in the *Rana* that have been studied the secretion is released in synchronous pulses which increase in frequency with temperature and also precede bouts of struggling by a restrained frog (Lillywhite, 1971b; Lillywhite & Licht, 1975). In *R. pipiens* the beads of mucous which appear on the dorsolateral folds are larger than those elsewhere on the back (pers. obs., frogs from Shirleys Bay; Table 3, July 1975). The skin of amphibians which are active in exposed diurnal environments is kept moist by the discharge of mucous from these glands, while fossorial, aquatic, and nocturnal species do not exhibit regular discharges (Lillywhite & Licht, 1975).

The skin secretions of various anurans have antimicrobial properties (Bachmayer *et al.*, 1967; Preusser *et al.*, 1975) and may protect the frogs from infection, perhaps especially at low temperatures when the amphibian immune system is inactive (Evans, 1963). Loss of the dermal mucous coat is a symptom of general ill-health in frogs (Gibbs *et al.*, 1966), and an antimicrobial function has been suggested for intestinal mucous produced during hibernation (Gossling *et al.*, 1980).

All amphibians lose water by evaporation at about the same rate as a free water surface, and

it is a general principle that more terrestrial species have thinner skins through which they achieve more rapid rehydration than aquatic forms (Schmid, 1965; Christensen, 1974). One selective advantage of reduced 'dermal gland area' is, thus, reasonably clear: it increases the area of thinner skin and enhances the rate of rehydration of a dehydrated frog, and so would be expected in more exclusively terrestrial populations. This relationship places *Rana pipiens* in an adaptive dilemma, however, since it is largely terrestrial during the summer but winters under water. Aquatic amphibians have thick skin, relatively impermeable to water (Schmid & Barden, 1965) and ions (Greenwald, 1972), so they do not absorb water or lose salts when they are in the water; thick-skinned *Rana* (including *R. pipiens*) survive 3-10 times as long as thin-skinned hylids and *Bufo* in distilled water (Schmid, 1965).

These relationships suggest three opposed functions for increased 'dermal gland area' in *R. pipiens*: 1) areas of enlarged glands may be a developmentally simple way to produce thicker, less permeable skin as an adaptation to a more aquatic local niche, 2) enlarged glands may serve to maintain the flow of water to the outside of uniformly impermeable skin as an adaptive response to dry habitats, or 3) the products of the glands may inhibit microbial infection. In the first case the area of enlarged glands should be correlated with the areal extent of water in the frogs' habitat, and in the second case with the aridity of the climate and the extent of dry habitats. The third alternative probably makes no geographic prediction distinct from the first.

Geographic Variation in Dermal Attributes of North American *Rana*

Rana aurora ranges from Vancouver Island to northern Baja California. In the north it is a pond and woods frog and often ranges far from water (Carl, 1959), whereas in California it is found on the vegetated banks of streams and in ponds, marshes, and reservoirs (Zweifel, 1955). The northern populations are smooth-skinned, large-legged, large-eyed, and long-headed, with narrow dorsolateral folds, a conspicuous face mask, and a dusky venter; their habitat is coastal rainforest. The southern populations are rough-skinned, with broader dorsolateral folds, shorter head and legs, less of a face mask pattern, conspicuous leg barring, and tendencies for ventral mottling and for dorsal spots to have a light centre; they are found along the margins of bodies of water in chaparral, dry forest, and grassland (Boulenger, 1920; Wright & Wright, 1949; Stebbins, 1966).

Rana sylvatica is widespread in northern North America. It is restricted to moist forests in the southern part of its range (Heatwole, 1961), but also frequents forest edges, meadows, wetlands, and pond and stream edges in the north (Schueler, 1973). There are marked east-west differences, and Martof and Humphries (1959) considered these to denote descent from stocks from separate glacial refugia. The eastern stock has more patterned, more rugose (which in this case means 'more glandular' in the terms of my discussion of leopard frogs) hindlegs, and the western stock a more patterned, more rugose trunk. In the boreal forest many western traits seem to have moved eastward (Martof & Humphries, 1959). The western populations characteristically have a light middorsal line (homologous to one of the middorsal stripe morphs of African ranids; the middorsal band of Palearctic *Rana* is homologous to the other; Boulenger, 1920). Fishbeck and Underhill (1971) and Schueler and Cook (1980) have correlated the frequency of the middorsal line with the grassiness of the habitat (at mid-continent), but there is some evidence that other factors may influence its distribution (Schueler & Cook, 1980).

Rana clamitans is a stream and pond-edge frog of eastern North America (Wright, 1914). The most striking feature of its colour variation is a break in the variation at the Fall Line of the Atlantic Coastal Plain (Mecham, 1954). Northern *R. clamitans* have much green on the face, variable, but often extensive, green on the anterior back, and the males have yellow throats; on the Coastal Plain this green and yellow is reduced or absent. Florida and Georgia frogs are especially heavily mottled ventrally; this mottling is most intense on young frogs, and at least some from as far north as coastal New Jersey have this ventral pattern (NMC specimens). Dark dorsal spotting is absent in Coastal Plain populations and increases gradually to the north. The dorsal skin of Coastal Plain frogs is less rugose than that of frogs from elsewhere (Mecham, 1954).

Rana boylei is a stream-dwelling frog of the coast and Sierra Nevada of California and Oregon. It shows a northward cline of darker dorsal ground colour, reduced dorsal spotting, and reduced distinctness of mottled patterning in the groin. The coastal populations have less

dark dorsal spotting than the montane ones, and south of San Francisco Bay there is a region where the usually bold throat mottling is suppressed (Zweifel, 1955).

The burrow-dwelling frogs of the *Rana pipiens* group (*R. areolata, s. l.*) have variously been considered one species (Conant, 1975), or many (Wright & Wright, 1949). The following summary is based on descriptions of the nominal taxa of which *R. areolata s. l.* is composed. There is a northward increase in 'wart'-like dermal glands in both the west (*R. circulosa* > *areolata s. s.*) and the east (*R. capito* > *aesopus* & *sevosa*). Frogs of the Atlantic and Gulf Coastal Plain (*R. sevosa* & *capito*) have heavily pigmented venters, those of the Mississippi drainage and adjacent Texas are largely unmarked ventrally, and those from Florida are intermediate. The western populations (*R. areolata* & *circulosa*) have dark spots outlined by light rings on a dusky ground, but the spots of the eastern populations are not outlined. The dorsal ground colour ranges from very pale in Florida to nearly black in Alabama and Mississippi.

Rana palustris is the most abundant member of the *R. pipiens* complex in the Appalachians, New England, and Maritime Canada; it is infrequent in much of the rest of eastern North America (Pace, 1974; Bleakney, 1958a). There are more or less concordant geographic steps in six aspects of its pigmentation which led Schaaf and Smith (1970) and Hardy (1964) to divide the species into an upland northern form, and a southern, Coastal Plain form, connected by intermediate and variable populations, often found in cave areas. The southern form has reduced dorsal spotting (fewer spots, snout spot less frequent, spots less square), frequent fusion of the dorsal spot primordia into longitudinal stripes or transverse blotches, dark ventral mottling, and pigmented vomerine teeth. It inhabits swamp forests.

Rana palustris has a very high density of serous glands, a characteristic which may be related to its notorious toxicity to snakes (Cott, 1940). Occasional individuals have fewer glands than is usual, but there does not seem to be any gross geographic variation in this attribute within the range of Schaaf and Smith's 'northern' and 'intermediate' forms. Gland characters did not load heavily in a weighted separation index between frogs from these regions in the NMC and AMNH collections (Schueler, unpublished).

Pace (1974) noted geographic variation within *Rana utricularia* in characters that otherwise tend to separate species of leopard frogs: the white tympanum spot, the presence of a snout spot, and ventral duskiness. Florida frogs have extreme values for all characters: 91% have a light tympanum spot, 2% have a snout spot, and ventral duskiness is general in the Keys and frequent elsewhere in Florida. Tympanum spotting decreases northward to 55% along the Atlantic Coastal Plain and to 40% in the Mississippi drainage, and the frequency of snout spotting increases northward to 15-20% at the limits of the range, though low values extend further north along the coast than in the interior. Extreme ventral duskiness is infrequent outside of Florida.

Because of the geographic morphological stability of *Rana pipiens* relative to variation among species of leopard frogs and within at least *R. utricularia* (Pace, 1974), there is little mention of geographic variation in the coloration or morphology of *Rana pipiens* in the literature of geographic variation among leopard frogs (see Moore, 1944). The exceptions to this rule are studies of the ecological genetics of two colour pattern morphs found in Minnesota and adjacent states. These are the burnsi and kandiyohi morphs, described as species (Weed, 1922) and since found to result from unlinked dominant alleles (Merrell, 1973). In burnsi dorsal spots are typically absent and epidermal melanocytes are more evenly dispersed than in spotted frogs. In kandiyohi the interspot areas of the back are irregularly mottled by light areas similar to the light rings around the spots of 'wild-type' frogs, and dermal and epidermal melanophores are more clumped than in the wild-type (Smith-Gill, 1975).

The distribution of burnsi is centred on the Anoka Sand Plain, a glacial outwash plain formed by the glacial damming of the Mississippi River, and that of kandyohi is centred on the prairie regions of Minnesota and the eastern Dakotas. Each makes up about 7% of the populations where it is most frequent (Merrell, 1965; McKinnell and Dapkus, 1973). Merrell (1965) noted that within the area where burnsi occurs the wild-type spotted frogs have about 1-2 fewer spots than they do elsewhere nearby, but what is more remarkable is that within this area of depressed spot number there is a positive correlation between the number of spots on the spotted frogs and the frequency of burnsi, so that the spotted frogs have about 13.2 spots where

burnsi is present but very rare, and 14.7 spots where the frequency of burnsi is 7% (r=.531, p<.01; for 26 counties where *burnsi* occurs and 100 or more frogs were sampled, weighted by number of frogs; data from Merrell, 1965, Tables 1&2).

There are three apparent burnsi in the sample of skins from the tip of Long Point (Schueler, 1979, 1981a&b). Wright and Wright (1949) found occasional unspotted *Rana pipiens* in commercial shipments from Vermont, and Anderson and Volpe (1958) mentioned reports of unspotted frogs from 'Maine' and 'Nevada'. There are 10 dorsally unspotted adult *R. pipiens* from St. Croix, Woodland, Maine, in the MCZ collection which were "selected from 36,000 frogs of which 96% were typical or intermediate" (label of MCZ 25541-50) by James Miller in May, 1940. These have some leg spotting and a few have small dark flecks on the back; they look very much like burnsi. These are apparently the only records of unspotted *R. pipiens* beyond the upper Midwest of the United States (Merrell, 1965). Unspotted *R. utricularia* have been found in New Jersey, southern Illinois, eastern Mississippi, Alabama, and Kentucky, generally in sandy areas (Brown & Funk, 1977), and unspotted *R. berlandieri* have been found in the lower Rio Grande valley (Sanders & Smith, 1971).

Casual reports of kandiyohi (as in Anderson & Volpe, 1958 and Sanders & Smith, 1971) are hard to evaluate, because there is a variety of mottled pattern variants in leopard frogs. There is one apparent kandiyohi among the skins from the tip of Long Point (Schueler, 1979, 1981a&b). Browder (1968) described a 'speckled' morph with irregular reduction in numbers of visible guanophores and xanthophores in the dorsal skin, so that dermal melanophores show through and produce a mottled pattern. These frogs were from shipments said to originate at the southern end of Lake Manitoba. Another similar form is what Merrell called 'pseudokandiyohi' (Merrell, 1965, Figure 5; and (*fide* Merrell) Wright & Wright, 1949, plate CIII, lowest figure), which appears similar to frogs I have examined from the Canadian prairies. In these there is dark mottling in interspot areas of the epidermis. The intensity of the markings is variable and the pigmentation seems to be in the stratum corneum of the skin (NMC specimens). There were 9 occurrences of this pattern among 204 specimens from southwestern Manitoba, southeastern Saskatchewan, and North Dakota that I examined in this study, and none among the 1488 specimens from elsewhere (Figure 26; Schueler, 1979, Figure I-5). Northern Great Plains populations of other large amphibious poikilothermic vertebrates, the Painted Turtle, *Chrysemys picta*, (Smith *et al.*, 1969, Schueler, in prep.) and the Tiger Salamander, *Ambystoma tigrinum*, (Gehlbach, 1967) also often have a dark-mottled dorsal pattern, so such a pattern may be cryptic on prairie pond bottoms.

As noted above, Merrell (1972) found that in the laboratory kandiyohi individuals metamorphose earlier than wild-type, and Merrell and Rodell (1968) found that burnsi individuals are over-represented in the survivors of overwintering, so the selective significance of these colour morphs, like those of *Plethodon cinereus* (Lotter & Scott, 1977) and *Rana arvalis* (Ishchenko & Shchupak, 1974) is compounded of visual and physiological factors.

Coloration of North American *Rana*

To summarize colour variation in *Rana* in North America I derived 14 quantitative multistate characters from 46 descriptions of the coloration of 24 species of North American *Rana* in Wright and Wright (1949), Conant (1958), H.M. Smith (1956), Mecham (1954), and Mecham *et al.* (1973). These characters do not directly describe dark dorsal pigmentation, which I scored for each species on the basis of Wright and Wright's descriptions and photographs (Table 2). Ordination of the 14 characters (excluding Dark Dorsal Pigmentation) by principal components analysis (PCA) of correlations gave the three-dimensional pattern plotted (with the dark dorsal pigmentation estimates) in Figure 1. Nonmetric multidimensional scaling of average taxonomic distance (NT-SYS programme; Sneath & Sokal, 1973) showed the same general pattern, but had more overlap between species groups. This is a picture painted in very broad strokes, because of the irregularity of the data, but it is a generally clear picture. PC1 (28% of the total variation) is a contrast between the aquatic *R. catesbeiana* group (Wallace *et al.*, 1973; Case, 1978) and the other species, yet among the leopard frogs and the western *Rana* the species are arrayed in approximate order of increasingly terrestrial habits: *R. fisheri=tarahumarae, utricularia, pipiens=berlandieri, blairi, onca*; and *pretiosa*, southern *aurora*, *cascadae*, *boylei*, and northern *aurora*, so the aquatic-terrestrial contrast seems to be an ecological rather than a phylogenetic

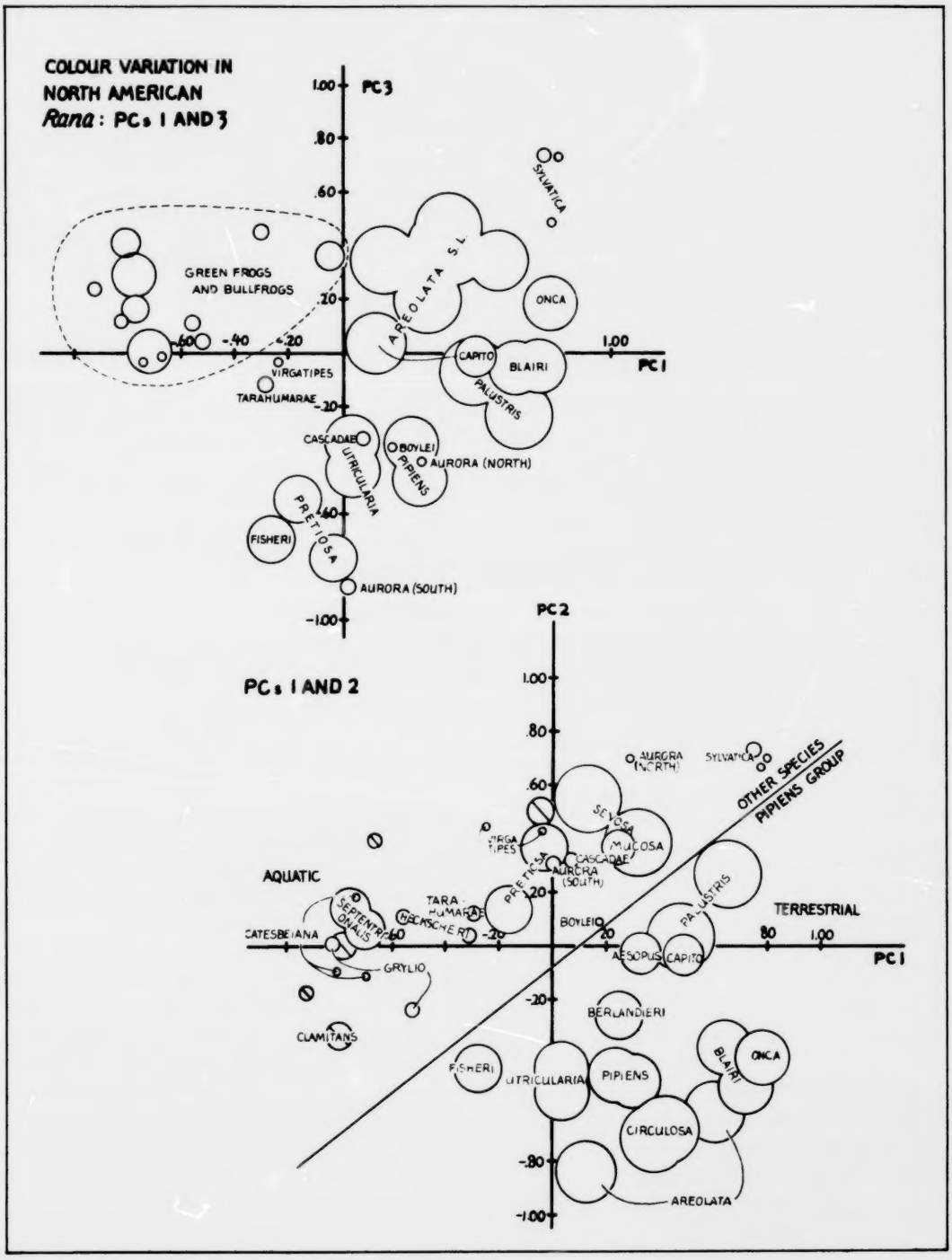

Figure 1. Colour variation in *Rana*: PCs 1-3.

These show the positions of colour descriptions of North American *Rana* on the first three PCs of their variation. The diameter of the circles is proportional to the species' score for DARK DORSAL PIGMENTATION, which was not included in the analysis. In PCs 1&2 five slashed circles represent descriptions of *R. clamitans*; in PC 1&3 'Green frogs and Bullfrogs' refers to *R. clamitans, R. catesbeiana, R. grylio,* and *R. hecksheri.*

Table 2. *Rana* coloration characters.

TYMPANUM CENTRE	0) tympanum unicolour 1) concolour with back 2) lighter than back
TYMPANUM PERIPHERY	0) concolour with back 1) darker than back
FACE MASK	0) absent 1) present
UPPER LIP	0) concolour with back 1) lighter than back
MALE THROAT COLOUR	0) not yellow 1) yellow
DORSAL GROUND COLOUR	0) green absent 1) yellowish or olive green 2) bright green
DORSAL GREEN	0) absent or restricted to head 1) anterior part of back 2) generally over back
ASHY-COLOURED FORMS	0) ground colour always dark 1) ground color sometimes ashy gray-tan
LIGHT OUTLINING OF DARK DORSAL PIGMENTATION	0) dark areas absent or not outlined 1) dark areas outlined
REDDISH DORSAL COLOUR	0) absent 1) bronzy 2) reddish
LIGHT JAWLINE	0) absent 1) present
VENTRAL GROUND COLOUR	0) white 1) pale yellow 2) bright yellow 3) orange 4) red
VENTRAL PATTERN	0) immaculate white 1) facultatively dusky 2) faintly or lightly mottled on throat 3) faintly or lightly mottled on most of venter 4) entirely mottled beneath
DORSO-LATERAL FOLD	0) darker than back 1) concolour with back or absent 2) somewhat lighter than back 3) white
DARK DORSAL PIGMENTATION	0) absent 1) flecks or few smudges 2) few spots or large smudges 3) flecks or mottles over much of the back 4) many spots 5) abutted spots or dark-mottled background

one. Aquatic species are green, have the upper lip lighter than the back, and yellow throats in the males, while terrestrial species have a light jaw line and ashy-coloured (pale tan) forms.

The second PC (18% of the variation) separates most of the frogs of the *Rana pipiens* group from the other species, especially the 'brown frogs' (*R. aurora* and *R. sylvatica*). Since this separation is based on light tympanum spots, spot outlining, and unpigmented venters, *R. palustris* and especially *R. sevosa* have higher values than the other species of the *R. pipiens* group.

PC3 accounts for 12% of the total variation in separating those species which have red or yellow ventral pigmentation, dorsal green, a jawline, and light dorsolateral folds from the other species: *Rana aurora*, *R. pretiosa*, and Wright and Wright's description of *R. fisheri* have the extreme values on this axis.

Part II: Variables — Methods & Materials

This Part describes analytic methods, data collection, and preliminary analyses used to reduce the dimensionality of the original data and to set up comparisons with external patterns of variation.

Methods of Analysis

I have used an array of parametric multivariate statistical procedures which can be thought of as creating new variables from the data so that the difference (measured as the sum of squared deviations) between the new variable and the original data is minimized (Pimentel, 1978; Cooley & Lohnes, 1971). In analysis of variance (ANOVA) the dependent variables are the groups into which the observations are divided and the new variables are the group means. In discriminant functions analysis (DFA) the groups are the dependent variables and new variables are compounded from more than one independent variable to minimize the variation within the groups, in effect the 'best' ANOVA. In multiple regression one variable is dependent and the new variable is the linear combination of the independent variables which best approximates the dependent variable. In principal components analysis (PCA) the variation that is minimized is that of the original data from the new variable, and in canonical correlations analysis (CCA) it is the difference between new variables created from two sets of data for the same objects. The extent to which the new variables simplify the structure of the data is expressed as the reduction in the variance of the data effected by measuring the variation as deviations from the new variable rather than on the original scale. This is the variation 'explained' or 'accounted for' by the analysis; it is the square of the correlation between the variables. The procedures used here to generate more than one new variable do so orthogonally, so each new variable is uncorrelated with all the others and varies independently of them among the observations on which the analysis is based. Most of the programmes I used generate standardized variables, which have a mean of zero and a standard deviation (and variance) of one among the specimens in the original analysis. Thorpe (1976) and Gould and Johnston (1972) have reviewed the use of multivariate methods in the study of geographic variation.

One can also combine characters to express variation among objects in a reduced number of dimensions by imposing an *a priori* structure which creates composite characters from the raw data. There are disadvantages with both methods: an *a posteriori* vector (such as a DF or PC) which 'explains' much of the variation among organisms may be composed of a jumble of characters with no relation to biological theory, while an *a priori* composite character such as an hybrid index may well not express the patterns of covariation among the original characters. If there is agreement between the patterns of variation shown by *a priori* and *a posteriori* characters, on the other hand, the hypotheses on which the *a priori* characters were based are corroborated, and the interpretation of the *a posteriori* characters is strengthened.

I have not dealt quantitatively with the assumptions of multivariate normality and homoscedasticity of the data associated with the statistical procedures, as they are difficult to test and the procedures have proven to be insensitive to many minor violations (Oxnard, 1978). I am seeking marked qualitative patterns in the data rather than trying to exhaust any supposed statistical 'significance' of variation, so I deal only with the first few axes of variation, which may, in many cases, be all that are at all repeatable (A.R. Gibson, pers. comm.). Most of the computations were done with two packages of statistical programmes: SPSS Version 7 (Nie *et al.*, 1976) and NT-SYS (Rohlf *et al.*, 1974; see Table 1).

Trend Surface Analysis

There are two ways to assign values to a variable at points in a plane defined by two other variables, so that a contoured map can be drawn of the first variable: either some or all of the data values can be averaged for each point, weighting in some way for distance, or an equation can be constructed which expresses the dependent variable as a function of the independent variables. The first method has the advantage that a 'possible' value is assigned to each point, as there is interpolation but no extrapolation, whereas the

second method has the advantage that the complexity of the surface is expressed by the complexity of the equation (Crain, 1970).

Trend surface analysis (TSA) is the stepwise inclusion of two variables (here latitude and longitude) and their powers and products (which I call 'geographic variables') as independent variables in a regression of a third variable by the exponential order of the terms (Marcus & Vandermeer, 1966; Gross, 1977). In the first (linear) step the dependent variable is regressed on X and Y, in the second (quadratic) step on X, Y, X**2, X*Y, and Y**2, in the third (cubic) step on X, Y, X**2, X*Y, Y**2, X**3, X**2*Y, Y**2*X, and Y**3, and so forth. The first step fits a plane surface to the data, the second a surface with a single fold, and subsequent surfaces are increasingly complex (see Figures 15-20 and 25).

To obtain multivariate trend surfaces which are expressions of independent kinds of geographic variation in all the data I have used CCAs of the specimen variables and geographic variables (TSA terms). This produces a few geographic patterns of defined complexity to which the variation of attributes can be compared. If, for example, all attributes had linear relationships with geography two (*e.g.*, north-south and east-west) patterns would suffice to describe all of the geographic variation and the variation in any attribute could be described by its correlations with these two patterns.

Path Diagrams

Path 'analysis' is the display of a model of causal relationships among variables as multiple regressions of dependent variables on variables that are held to affect their values. This can help to clarify overlapping relationships among complexly related variables by displaying the direct and indirect influences of the variables on each other. The measure of the strength of the relationships is the standardized regression coefficient (the change in the dependent variable produced by a unit change in the independent variable when both are measured in standard deviations). In this context these are called 'path coefficients' (Kerlinger and Pedhazar, 1973).

Figure 2 shows the arrangement of the environmental variables for path diagrams of the skin data. The arrow widths are proportional to the path coefficients and the area of circles to the unexplained variation. I have considered geographic and altitudinal position, topographic relief and lake and river size to be independent variables. I set climate dependent on position and the habitat variables dependent on elevation, relief, climate, and LAKE and RIVER SIZE (see Table 10 and Figure 14). In the diagrams in Part V the skin variables are considered to be dependent on latitude, climate, LAKE, RIVER, and WETLAND SIZE, and the habitat variables.

Names of Variables

I call attributes taken directly from specimens or maps 'characters' and call transformations and combinations of characters 'variables'. The names of variables are made of a wholely capitalized verbal description of the meaning of the variable and/or an abbreviation of the analysis in which it originated: thus TIBIA BROKEN BARS+MSF5 is the name of the fifth factor of the *M*ulti*S*tate *F*actor analysis, high values of which indicate the presence of broken bars in the pigmentation of the tibia, and SPOTTING-DF1 refers to the general spotting variable as the first discriminant function among localities, low values of which (as shown by the negative sign) indicate extensive overall spotting. Highly correlated variables may have the same verbal description, as LINEATION-CC3 and LINEATION+MSF3. The analyses abbreviated in variable names are the principal components analysis of climate (PC), the discriminant functions among localities (DF), the factor analysis of the multistate characters (MSF), and the canonical correlations analysis between the skin and environmental variables (CC). In tables and figures I sometimes use unique truncations of variable names. Attributes named in lower case refer to more than one variable: thus 'DORSAL SPOTTED' is a grid variable, but 'tibia spotting' is a generalization about variables that describe spotting on the tibia.

Samples of Skins

I collected frogs, by hand or with a light net, in meadows or at the edges of bodies of water, from 1971-1974. Localities and sample sizes are listed in Figure 3 and Table 3. Of 828 skins, 407 were female and 418 were male (sex data lost for 3), but the sex ratios of the samples varied widely, depending upon the season and circumstances of collection. I killed frogs in alcohol or by freezing. Freezing is less satisfactory as the outer layer of

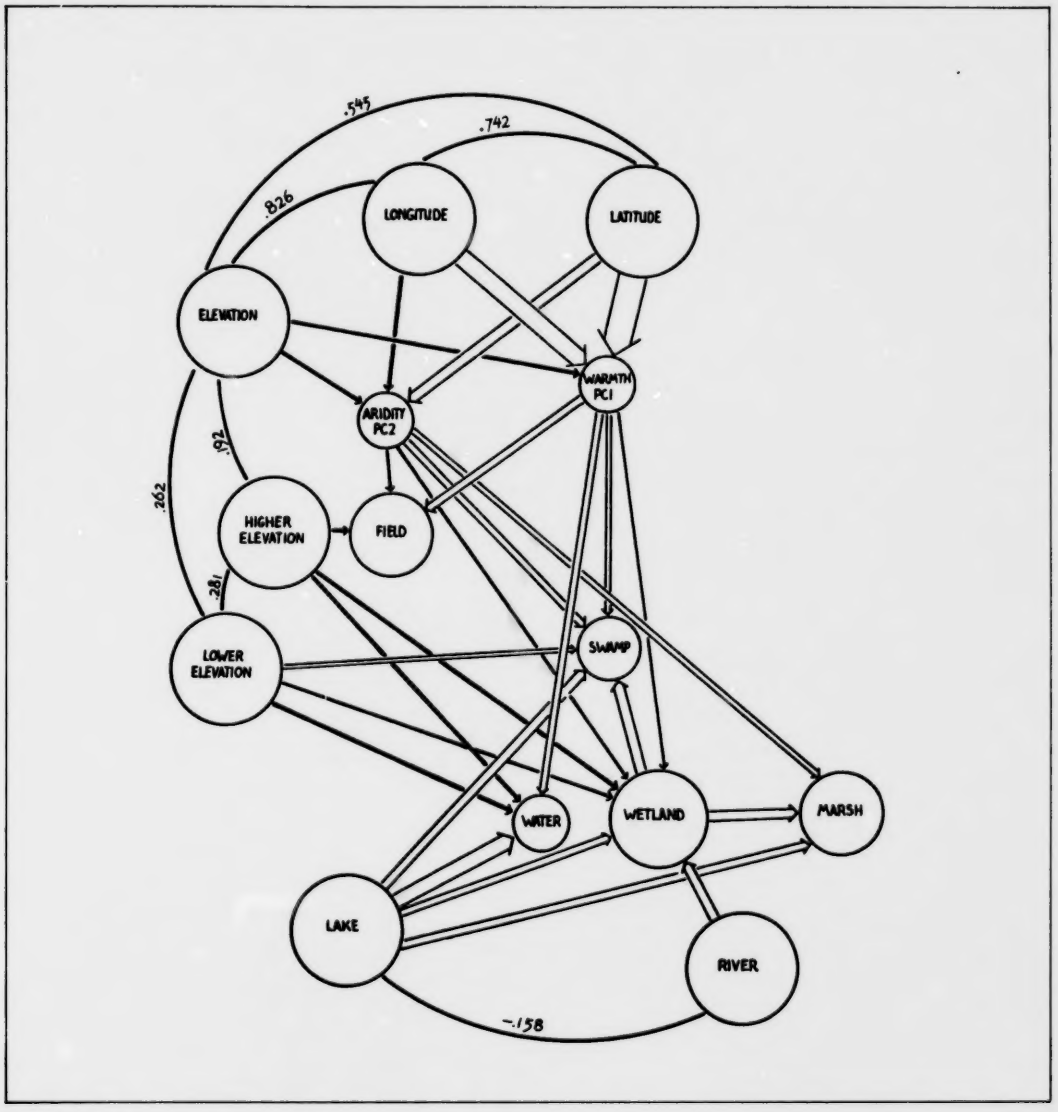

Figure 2. Path diagram of habitat variables.

This diagram represents an arrangement of environmental variables (pp. 24-30) in which variables are regressed on other variables considered causally prior to them. The independent variables (LATITUDE, LONGITUDE, ELEVATION, HIGHER & LOWER ELEVATION, and RIVER WIDTH represent the position and topography of the sites; these are held to influence the climate and habitats as shown in the diagram. The area of the circle for each variable represents the variation not explained by the regression, and is proportional to the area of the circles of the independent variables. The width of the arrows is proportional to the path coefficients. Curved lines and associated numbers show correlations between independent variables. The path coefficients are:

LATITUDE	→ WARMTH	-1.312	WARMTH	→ FIELD	0.368	HIGHER ELEV	→ FIELD	0.206
LONGITUDE	→ WARMTH	0.904	LAKE	→ WETLAND	0.333	HIGHER ELEV	→ WETLAND	-.202
LAKE	→ WATER	0.662	WARMTH	→ WATER	-.331	HIGHER ELEV	→ WATER	-.197
LONGITUDE	→ ARIDITY	0.596	ARIDITY	→ SWAMP	-.272	LATITUDE	→ ARIDITY	0.170
WETLAND	→ SWAMP	0.589	ARIDITY	→ MARSH	0.268	LOWER ELEV	→ WETLAND	0.160
LAKE	→ SWAMP	-.500	WARMTH	→ SWAMP	0.243	ELEVATION	→ ARIDITY	0.159
WETLAND	→ MARSH	0.468	LOWER ELEV	→ SWAMP	0.242	ARIDITY	→ WETLAND	-.159
RIVER	→ WETLAND	0.420	ELEVATION	→ WARMTH	-.236	ARIDITY	→ FIELD	0.128
LAKE	→ MARSH	0.369	LOWER ELEV	→ WATER	-.210	WARMTH	→ WETLAND	0.116

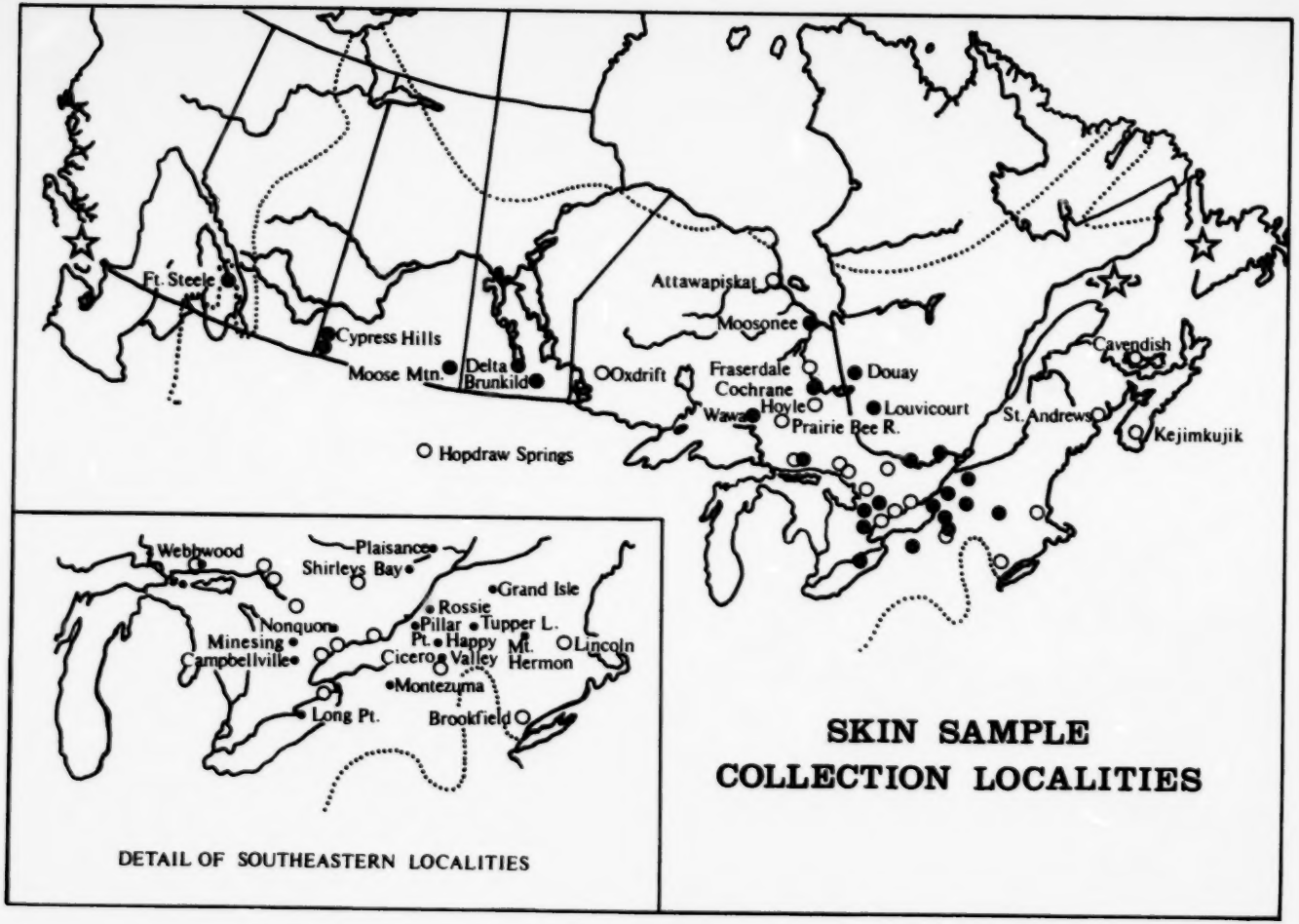

Figure 3. Skin sample collection localities.

Solid circles indicate sites where 10 or more specimens were taken. Open circles (not labelled in southern Ontario) indicate smaller samples. Collection data are in Table 3. The dotted line is the range limit of *Rana pipiens* (Pace, 1974; Conant, 1975). Stars indicate introductions (Cornerbrook, Newfoundland (Buckle, 1971 & Maunder, in prep.; Anticosti Island (Johansen, 1926; Hamilton Marsh, Vancouver Island (Green, 1978)).

Table 3. Collection data for *Rana pipiens* skins.

Locality Name	Specimens (males, females)	Location latitude	longitude
LARGER SAMPLES (n>10, used in among-localities DFA)			
Mount Hermon Massachusetts: Franklin Co.: Connecticut River valley between US Hwy 10 & Mt. Hermon School.	(30,12)	42.686°N	72.468°W
Grand Isle Vermont: Grand Isle Co.: 2 km N South Alburg on Hwy 2.	(16,13)	44.906	73.274
Tupper Lake New York: St. Lawrence Co.: Tupper Lake near Raquette R.	(5, 8)	44.203	74.472
Plaisance Quebec: Ottawa R. E of Plaisance: Parc Dollard des Ormeaux.	(11,11)	45.603	75.156
Rossie New York: St. Lawrence Co.: 0.75 km W Rossie.	(4,20)	44.637	75.639
Shirleys Bay Ontario: Carleton Co.: Shirleys Bay, 3 km NNW South March.	(3,18)	45.378	75.909
Happy Valley New York: Oswego Co.: Happy Valley Game Management Area.	(38,16)	43.458	76.000
Cicero Swamp New York: Onondaga Co.: Cicero: Cicero Swamp.	(8, 4)	43.167	76.042
Pillar Point New York: Jefferson Co.: Pillar Point.	(21,12)	43.975	76.167
Montezuma New York: Seneca Co.: Montezuma Marshes.	(32,23)	42.983	76.767
Louvicourt Quebec: Abitibi Co.: 4 km N Louvicourt.	(10, 4)	48.101	77.397
Douay Quebec: Douay Township: mile 80 on Hwy 61 N of Amos.	(12, 2)	49.517	78.019
Nonquon River Ontario: Ontario Co.: Nonquon R. 6 km NW Port Parry.	(18,25)	44.037	78.979
Minesing West Ontario: Simcoe Co.: Minesing Swamp, 6 km N Angus.	(4,15)	44.386	79.883
Minesing East@ Ontario: Simcoe Co.: Minesing Swamp, 8 km S Minesing.	(0, 1)	44.367	79.836
Campbellville Ontario: Halton Co. Forest Preserve: 6 km NNW Campbellville.	(17,10)*	43.518	79.997
Tip of Long Point Ontario: Norfolk Co.: Long Point: tip of the point.	(12,18)	42.561	80.058
Gravelly Bay Long Pt.@ Ontario: Norfolk Co.: Long Point: S of Gravelly Bay.	(4, 4)	42.556	80.106
Base of Long Point Ontario: Norfolk Co.: Long Pt.: 2-3 km E of the causeway.	(13, 8)	42.583	80.417
Moosonee Ontario: Cochrane Dist.: Moosonee.	(11, 5)	51.278	80.635
Cochrane Area Ontario: Cochrane Dist.: vicinity of Cochrane.	(4, 8)	49.0	81.0
Webbwood Ontario: Sudbury Dist.: 1 km E Webbwood.	(18,16)	46.275	81.871
Wawa Ontario: Algoma Dist.: 10 km ENE Wawa, Firesand Creek at Hwy 101.	(4, 9)	48.315	84.558
Brunkild Manitoba: 1.5 km E Brunkild.	(18,13)	49.583	97.533
Delta Manitoba: 4 km W Delta.	(21,29)	50.183	98.369
Moose Mountain Saskatchewan: Moose Mtn. Park: near White Bear Lake.	(25,31)	49.817	102.267
Cypress Hills South Saskatchewan: 18 km N Vidora: Frenchman R. at Hwy 21.	(7, 9)	49.483	109.367
Cypress Hills North Saskatchewan: 30 km S Maple Creek: ponds near entrance to Cypress Hills Park.	(10, 4)	49.658	109.494
Fort Steele British Columbia: 11.5 km N Fort Steele.	(12,21)	49.711	115.739

Table 3. Collection data for *Rana pipiens* skins. (Continued From Page 13)

Locality Name	Specimens (males, females)	Location latitude	longitude
SMALLER SAMPLES (n<10)			
Cavendish, P.E.I.	(1,0)	46.497 N	63.400 W
Kejimkujik, N.S.	(1,0)	44.408	65.250
Saint Andrews, N.B.	(1,0)	45.150	67.083
Saint Andrews, N.B.	(0,1)	45.250	67.117
Lincoln, Mass.	(2,3)	42.392	71.350
Brookfield, Conn.	(2,2)	41.433	73.400
Lake Onondaga, N.Y.	(1,0)	43.108	76.233
Palmers Rapids, Ont.	(1,3)	45.317	76.233
Napanee, Ont.	(4,3)	44.258	76.975
Duffins Creek, Ont.	(4,1)	43.817	79.033
Toronto Island, Ont.	(2,0)	43.617	79.383
Port Colborne, Ont.	(1,2)	42.839	79.278
Moon River, Ont.	(0,2)	45.056	79.767
Breakwater Long Pt., Ont.	(0,4)	42.561	80.286
Shawanga River, Ont.	(0,2)*	45.558	80.294
Point au Baril, Ont.	(1,3)	45.650	80.411
Massey, Ont.	(0,1)	46.244	81.931
Attawapiskat, Ont.	(1,3)*	52.875	82.292
Hoyle, Ont.	(3,3)	48.553	81.008
Fraserdale, Ont.	(2,3)	49.850	81.617
Prairie Bee R., Ont.	(0,1)	47.858	83.900
Oxdrift, Ont.	(3,0)	49.817	93.025
Hop Draw Spring, S.D.	(0,1)	43.733	103.967

@Pooled with the preceeding sample in the DFA.

* Plus one unsexed specimen.

the epidermis tends to slough off the skin, which obscures some characters. I cut each skin along the midventral line and along the midventral lines of the limbs, removed it from the frog, rinsed it in water, spread it between waxed papers with its field number afixed on a small paper label, and dried it in a plant press (more detail is given in Schueler, 1981b).

Differences in the way I prepared different groups of samples had no detectable influence on their scores for the major axes of variation among localities (Schueler, 1979).

Multistate Characters

These are descriptions or counts of the extent of dark pigmentation, light pigmentation, and dermal glands on the skins. They were scored by a combination of transmitted and reflected light. (Figures 4&5, Appendix 1).

To have fewer and more normally and continuously distributed variables to represent the multistate data, I computed PCs (SPSS FACTOR programme) of the correlations among the 31 multistate characters for all of the skins and rotated the first 18 PCs to a quartimax solution, which makes the factors as much as possible a combination of a few variables, so that these have loadings approaching one and the other characters have loadings approaching zero. The 18 factors accounted for 82% of the variation (equivalent to that of 25 characters), and only the first few factors accounted for much more variation than that attributable to a single original variable (Table 4). The 18 factors are:

SPOTTING+MSF1. This is an overall measure of how much pigmentation there is on the skin. It has high positive loadings from TYMPANUM OUTLINING, INTENSITY and EXTENT OF DARK JAWLINE, FEMUR AND LATERAL RETICULATION, JAW MOTTLING, and CANTHUS-ROSTRALIS LINE, and a large negative loading from FEMUR PALENESS. There are weaker positive loadings from merged dorsal spots, LATERAL SPOT LINEATION, dark FEMUR BACKGROUND, complete femur and tibia bars, and negative loadings from tibia glands and tibia and femur half bars. This factor is positively correlated with all of the grid and metric measures of spotting but is negatively

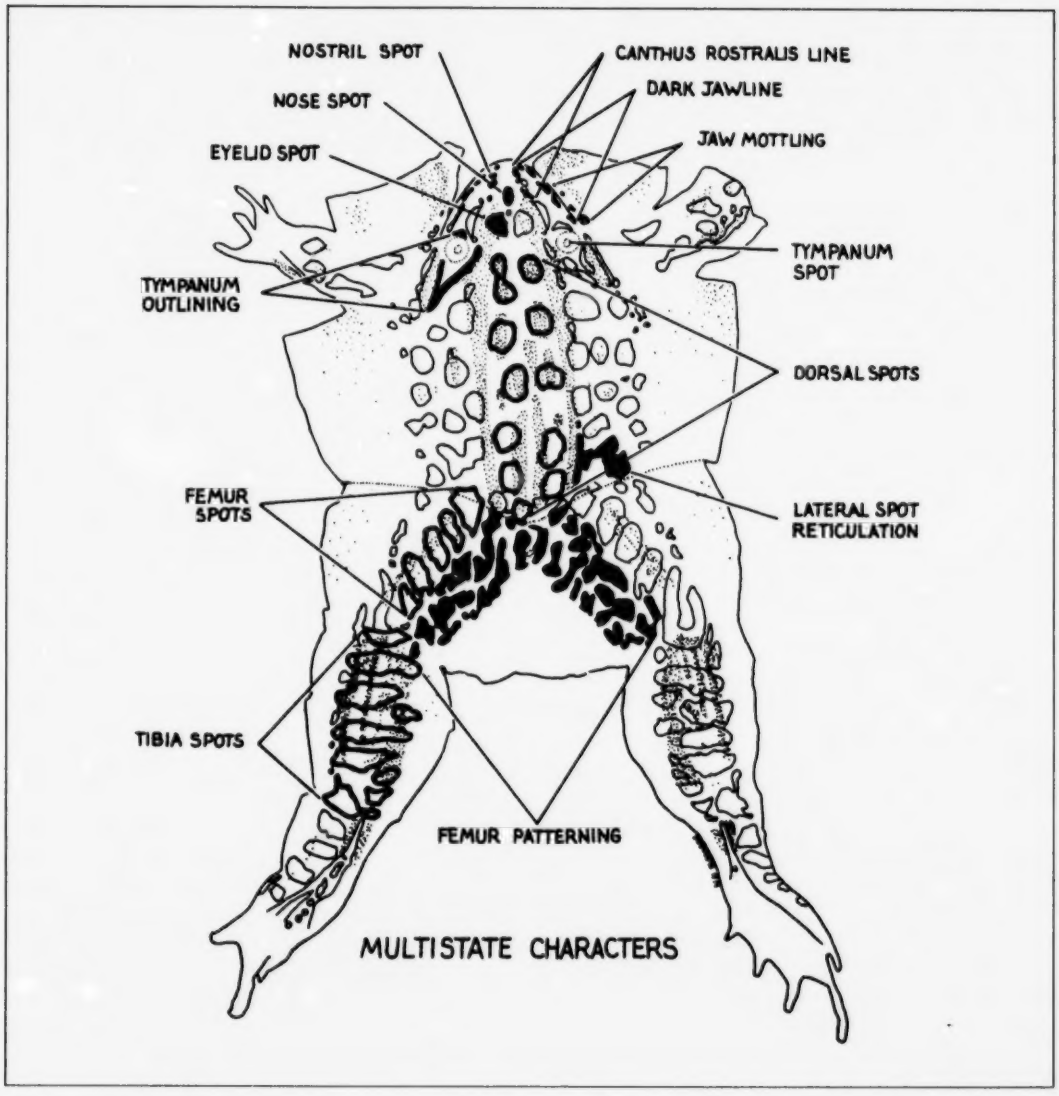

Figure 4. Multistate pigmentation characters.

Characters are listed in Appendix 1. The pigmentation described by each of the characters is heavily outlined or blacked in.

correlated with the NUMBER OF DORSAL SPOTS, and has a negative correlation (only among localities) with SKIN SIZE (Table 5).

VENTRAL GLANDS+MSF2. This has high loadings from the three ventral gland characters, and a weak negative loading from dorsolateral fold lightness. It is positively correlated with tibia gland area and with the length of the LONGEST GLAND LINE.

LINEATION+MSF3. This factor has its highest loading from the distinctness of the light lines along the dorsolateral folds and the jaw, and has lesser but still substantial contributions from the EXTENT OF DARK JAWLINE and the CANTHUS-ROSTRALIS LINE. It is not much correlated with any of the metric or grid variables (Table 5).

HEAD SPOTS+MSF4. This factor is positively correlated with dorsal spot number and area; it has high loadings from both eyelid spot characters and a weak loading from NOSE SPOTS.

Factors 5 to 8 and 14 contrast a leg spotting character with either all the other leg spotting characters or complete barring. There is no strong

Table 4. Eigenvalues of multistate factors.

	Factor	Eigenvalues, as characters*	
		Unrotated PCA**	Rotated Factors
1	SPOTTING	5.07	4.28
2	VENTRAL GLANDS	2.61	2.38
3	LINEATION	1.88	1.96
4	HEAD SPOTS	1.79	1.58
5	TIBIA BROKEN BARS	1.57	1.48
6	FEMUR DIAGONAL BARS	1.39	1.13
7	TIBIA HALF BARS	1.24	1.24
8	FEMUR HALF BARS	1.13	1.34
9	LIGHT TYMPANUM SPOT	1.07	1.02
10	SPOT OUTLINING	1.04	1.01
11	NOSTRIL SPOT	0.99	1.02
12	NOSE SPOT	0.97	1.05
13	DUSKINESS	0.87	0.95
14	FEMUR BROKEN BARS	0.86	1.03
15	DARK FEMUR BACKGROUND	0.82	1.06
16	MERGED DORSAL SPOTS	0.75	1.03
17	SPOT BILINEARITY	0.74	1.02
18	LATERAL LINEATION	0.73	0.98

*Each unit of the eigenvalues is equivalent to the variation due to one character in the original data. The first factor accounts for 14% of the total variation, the 18 factors account for 82% of the total.

**The PCs of which these are the eigenvalues do not necessarily correspond to the rotated factors in any way except sequential position.

relationship between the patterns of the two segments of the leg beyond that expressed in SPOTTING+MSF1. Most of these factors are little correlated with any of the grid or metric variables. They are: TIBIA BROKEN BARS+MSF5, rather than full bars, with a weak loading from FEMUR HALF BARS; FEMUR DIAGONAL BARS+MSF6; TIBIA HALF BARS+MSF7; FEMUR HALF BARS+MSF8, rather than full bars, with weak negative correlations with tibia spotted area and FEMUR MIDLINE SPOTTING; and FEMUR BROKEN BARS+MSF14.

None of the remaining factors is quite a unit vector, but I name each from the one character from which it has a high positive loading: LIGHT TYMPANUM SPOT+MSF9, with weak negative loading from CANTHUS-ROSTRALIS LINE; SPOT OUTLINING+MSF10, with weak negative loading from JAW MOTTLING; NOSTRIL SPOT+MSF11, with weak positive loading from TYMPANUM OUTLINING and weak correlations with NOSE SPOT LENGTH and dorsal spotting; NOSE SPOT+MSF12, with weak positive loading from most of the head pigmentation characters and positive correlations with NOSE SPOT LENGTH, NUMBER OF DORSAL SPOTS, and dorsal spot area; DUSKINESS+MSF13, with negative loading from JAW MOTTLING; DARK FEMUR BACKGROUND+MSF15, with positive loadings from LATERAL SPOT and FEMUR RETICULATION and a weak correlation with FEMUR LINE; MERGED DORSAL SPOTS+MSF16, with positive loading from LATERAL SPOT RETICULATION and negative from JAW MOTTLING, and positive correlation with spot area and the length of the LONGEST SPOT; SPOT BILINEARITY+MSF17 with weak negative loading from NOSE SPOTS, correlated negatively with the NUMBER OF DORSAL SPOTS and positively with the length of the LONGEST SPOT; and LATERAL LINEATION+MSF18, with weak negative loading from JAW MOTTLING.

Metric and Grid Characters

The metric characters are measurements, made to the nearest millimetre with a flexible opaque ruler by transmitted light, of areas of dark pigmentation, glands, or parts of the skin (Appendix 1, Figure 6). Three of these were included only as measures of the size of the skin (SKIN LENGTH, FEMUR MIDLINE, & TIBIA LENGTH).

I examined the skins, mostly by transmitted light, under a grid of points on clear photographic film. A 5.04 mm grid was used on the back and a 2.42 mm grid was used on the tibia. On the back (within the canthus-rostralis lines, the eye-

lids, and the midline of the dorsolateral folds) points were scored for the presence or absence of dark pigment. If one count was less than 12, the grid was shifted and the counts were repeated and averaged, so the total sample for each specimen was always based on (many) more than 35 points. The pigmented (dorsal) area of the tibia was similarly sampled, scored for both dark pigmentation and the presence of dermal glands conspicuously larger than those of the background condition (Appendix 1).

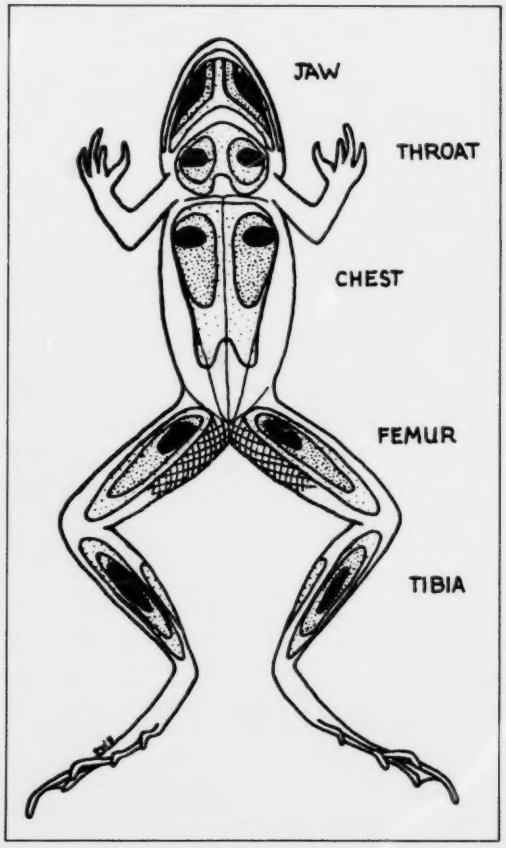

Figure 5. Multistate dermal gland characters.
This shows the area of glands corresponding to each character state of the ventral gland characters (Appendix 1) as they appear on an intact frog. Black=1, heavy stipple=2, and light stipple=3. The extent of glands on the chest and femur (not scored on the skins) appeared to be as highly correlated with overall gland extent as that on the jaw, throat, and tibia.

There was little ambiguity in scoring pigmentation, but the distinction between the concentrations of larger glands scored as glandular and the background incidence of smaller glands was not always sharp, especially in specimens with little glandulation where the larger glands were fairly small. Also, the background condition in very glandular specimens was as dense as the condition scored as enlarged in less glandular specimens, so these characters underestimate the difference between more and less glandular specimens. Dorsal glands were not scored because they were more often obscure than those of the tibia, with which they appeared to be highly correlated.

The square roots of the grid measurements (areas) were used to make them comparable with the linear measures of the metric data, and to equalize the variances of samples of different mean size the base 10 logarithms of these data (plus one, so that all values were defined) were used in the computations.

Skin Size

To measure the size of skins I used the specimen scores on the first PC of a PCA of correlations among base 10 logarithms of five variables that are expressions of the linear dimensions of the skin and do not measure glands or pigmentation: SKIN LENGTH, FEMUR MIDLINE, TIBIA LENGTH, and the square roots of the area of the back and tibia (as the sums of the dorsal and tibia grid counts). The zero points of the original and transformed variables were set equal by adding one to the data before transformation. This first PC accounted for 90% of the variance, and all of the variables were correlated with the eigenvector above 0.92 so it is a good measure of size (Blackith & Reyment, 1971). PCs 2 and 3 together accounted for 7% of the variance and together contrasted the metric and grid and back and leg variables.

A priori Composite Variables

I formed eleven *a priori* variables: six indices, two estimates of parameters (Table 6), two discriminations between the two species of frogs most similar to *Rana pipiens* of which I had skins, and a DF between the sexes of *R. pipiens*.

Discriminations between *Rana utricularia* and *R. palustris* were made to see how the patterns of difference between sympatric or parapatric species closely related to *Rana pipiens* varied geographically in *R. pipiens*. *R. utricularia* is the Southern Leopard Frog, and replaces *R. pipiens*

Table 5. Correlations between MSFs and grid and metric variables.

'=' indicates an absolute value less than 0.10; Factors (6, 9, 13, 14) and variables (in the lower part of the table) with no correlation greater than this are not listed.

	FACTOR							
	1	2	3	4	5	7	8	10
NOSE SPOT LENGTH	0.14	=	=	=	=	=	=	=
LONGEST GLAND LINE	=	0.37	=	=	=	=	=	=
LONGEST DORSAL SPOT	0.11	=	-.15	=	=	=	=	=
MID FEMUR SPOTTING	0.31	=	-.10	=	=	-.11	-.18	0.14
FEMUR LINE	0.58	=	-.10	=	=	=	-.11	=
TIBIA LINE	0.58	=	=	=	=	-.12	=	=
DORSAL UNSPOTTED	-.45	0.14	=	=	=	=	=	0.10
DORSAL SPOTTED	=	=	-.10	0.24	=	=	=	0.13
UNSPOTTED-NO GLAND	-.21	-.38	=	=	0.12	=	=	=
UNSPOTTED-GLANDS	-.38	0.47	=	=	=	=	=	=
SPOTTED-NO GLANDS	0.34	-.42	=	=	=	=	-.22	=
SPOTTED-GLANDS	=	0.36	-.16	=	=	=	=	0.13
NUMBER OF DORSAL SPOTS	-.13	=	=	0.30	0.12	=	=	=
SKIN SIZE	-.31	=	=	=	=	=	=	0.14

	FACTOR					
	11	12	15	16	17	18
NOSE SPOT LENGTH	0.14	0.78	=	0.11	-.11	=
LONGEST DORSAL SPOT	=	=	=	0.25	0.18	=
MID FEMUR SPOTTING	0.11	=	=	0.17	=	=
FEMUR LINE	=	=	0.17	0.10	=	=
DORSAL UNSPOTTED	=	-.12	=	=	=	=
DORSAL SPOTTED	0.14	0.13	=	0.17	=	=
TIBIA UNSPOTTED-GLANDS	=	=	0.11	=	=	=
NUMBER OF DORSAL SPOTS	=	0.34	=	=	-.37	-.11

in southeastern North America. *R. palustris*, the Pickerel Frog, is broadly sympatric with both species, but does not range as far north, south, or west as the leopard frogs. It is characteristically associated with streams and boggy places (Schaaf & Smith, 1970; Pace, 1974). *R. palustris* sometimes hybridizes with *R. pipiens* (Pace, 1974; Hardy & Gillespie, 1976; C.A. Campbell, in prep.) and perhaps with *R. utricularia* (Salthe, 1969).

I used 49 *Rana utricularia* skins, mostly from Florida and New Jersey, and 40 *R. palustris* skins, mostly from New York and New England, to compute a DF and a weighted separation index (WSI, Vogt & McPherson, 1972) between these species. The texture of the skin of the three species is so different that I was not able to score the grid characters on *R. palustris* or *R. utricularia* (in the former the glands are too indistinct on the tibia, and in the latter the dorsal spots are often indistinctly different from the background). I allotted multistate characters with defined character states to the WSI and the counts of dorsal, merged, femur and tibia spots and the metric characters (untransformed) to the DF. I used a two-group DF programme written by D.M. Power, based on Anderson (1966), and a WSI programme written by M.A. Gates.

The coefficients of the variables are tabulated in Tables 7 and 8. In both cases the reference species were easily discriminated and *R. pipiens* fell between them but closer to *R. palustris* (Figure 7). The WSI is based on the presence of snout spots and extensive dermal glands in *R. palustris*, and of light tympanum spots and light dorsolateral folds in *R. utricularia*. The DF has high loadings from NOSE SPOT LENGTH and a variety of leg spotting characters; *R. utricularia* has fewer full bars but more spots of other types on the femur and much less spotting on the tibia than *R. palustris*. *R. utricularia* averages somewhat larger than *R. palustris* (Pace, 1974), but the coefficients for the size characters are low, so size is not making much contribution to this discrimination.

Discrimination Between the Sexes

The ecological differences between the sexes of *Rana pipiens* are relatively well known (Dole,

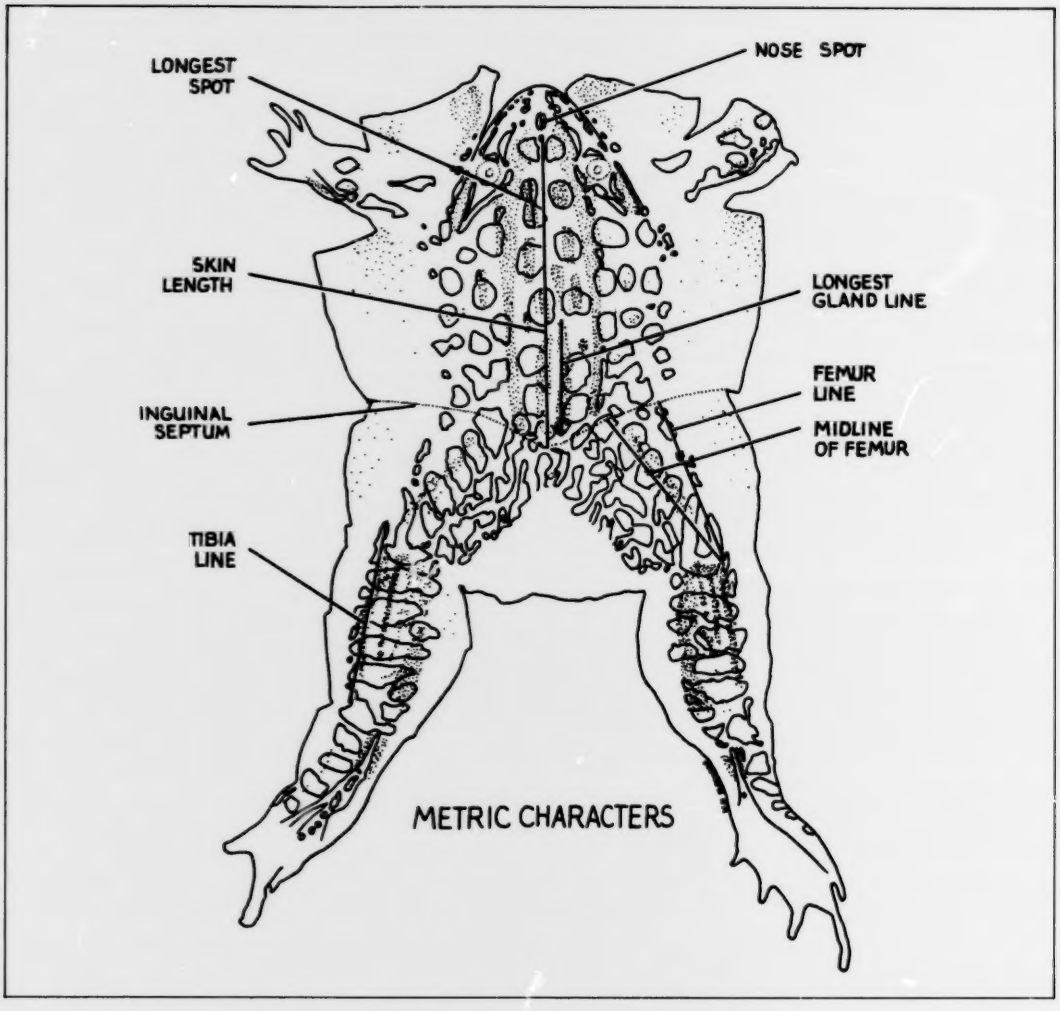

Figure 6. Metric characters.

The line segments show the measurements and the intervals along which FEMUR LINE, FEMUR MIDLINE SPOTTING, and TIBIA LINE were measured. The inguinal septum partitions the subdermal lymph space; it is evident on the dried skins as a translucent line. Characters are listed in Appendix 1.

1965; Wright, 1914), so, given the assumption that the populations that are more like one sex in colour pattern are more like that sex in ecology, a variable defined as maximizing the difference between the sexes allows speculation about the overall ecology of the populations. Male *R. pipiens* spend more time in the breeding ponds than the females (Merrell, 1968), and spend the summer in wetter habitat (Dole, 1965), so it seems plausible to predict that male leopard frogs should be coloured more like aquatic *Rana* than females, that is, with, fewer linear pattern elements such as the face mask and light jaw line emphasized by PC1 of the *Rana* PCA, and perhaps more ventral patterning and less dark dorsal pigmentation. Males average smaller than females (Wright & Wright, 1949), and since there are morphometric differences between the sexes (Schueler, unpublished) some differences in size and proportions might be expressed by a DF between the sexes.

Karlstrom (1962) has explained sexual dichromatism in toads of the *Bufo boreas* group in terms of similar habitat differences; males are less melanic and less patterned and spend more time at the breeding ponds, and this pattern is more marked in populations which breed more diurnally. Stewart and Kramek (1980) describe more

Table 6. Indices from multistate characters.

SUMMED FEMUR SPOTTING, SUMMED TIBIA SPOTTING: Twice the number of whole, broken, and diagonal bars, plus the number of half bars.

GLAND INDEX: The mean of the scores for jaw, throat, and tibia dermal glands.

COLORATION UNIFORMITY INDEX: The sum of the characters which express a lack of contrast within (FEMUR PALENESS) or between (DUSKINESS) the dorsal and ventral surfaces, minus those that express enhanced contrast between light and dark areas on the dorsal surface (SPOT OUTLINING, DORSO-LATERAL FOLD COLORATION, FEMUR BACKGROUND COLOUR, INTENSITY OF JAWLINE and EXTENT OF DARK JAWLINE).

LINEATION INDEX: The sum of the characters which represent lines or linear arrays of pigmentation (CANTHUS-ROSTRALIS LINE, EXTENT OF DARK JAWLINE, EXTENT OF LIGHT JAWLINE, SPOT OUTLINING, DORSO-LATERAL FOLD COLORATION, DORSAL SPOT BILINEARITY and LATERAL SPOT LINEATION).

RETICULATION INDEX: The sum of the scores for characters which express a tendency for dark pigmentation to be distributed as a continuous web or reticulum rather than as discrete spots (JAW MOTTLING, NUMBER OF MERGED DORSAL SPOT PRIMORDIA, LATERAL SPOT RETICULATION, FEMUR PATTERNING, TYMPANUM OUTLINING and CANTHUS-ROSTRALIS LINE/2).

SPOTTING INDEX: The sum of scores for characters that describe the extent of spotting (NOSE SPOTS, NOSTRIL SPOT, RIGHT EYELID SPOTS, LEFT EYELID SPOTS, TYMPANUM SPOT*-1, NUMBER OF DORSAL SPOTS/6, TYMPANUM OUTLINING/2 and FEMUR BACKGROUND COLOUR).

SPOT AREA: The average of the percentage of the dorsal and tibia grid points on spots.

GLAND AREA: The percentage of the tibia grid points on glands.

extensive dark, reticulated pigmentation of females of *R. septentrionalis*.

I computed the DF between the sexes (SEX-DF) using the variables used in the DFs among localities: 18 MSFs and 12 metric and grid variables, run on the SPSS DISCRIMINANT programme with the 418 male and 407 female skins.

SEX-DF (Table 9, Figure 8) agrees quite well with the predictions of the hypothesis that males are more aquatic. Females have more dorsal markings, especially on the head, and thus are more terrestrial by PC1 of the *Rana* PCA. Males have more lateral patterning (FEMUR LINE and LATERAL LINEATION), which is perhaps analogous to the bold ventral mottling of aquatic *Rana* (Schaaf & Smith, 1970; this did not show up in the PCA because wood frogs also are mottled below). In order of decreasing importance, females have more general SPOTTING, less FEMUR LINE, more LINEATION, less DUSKINESS, and more MERGED DORSAL SPOTS (all MSFs), shorter LONGEST SPOT and NOSE SPOT LENGTH, and more spotting on the dorsal grid. The dorsal and tibia grid characters have opposite signs, so males have a greater tibia area than females, which may reflect a longer bone, greater muscle mass, or a larger pigmented area. The dermal gland variables and the NUMBER OF DORSAL SPOTS do not differ between the sexes, nor does back colour (Schueler, 1979 and in prep.). When this discrimination is done using the raw multistate characters, the DF is dominated by the greater TYMPANUM OUTLINING of the females.

The DF correctly identifies 63% of both male and female specimens, but the sexual variation accounts for only 13.7% of the total variation in the SEX DF. ANOVA among localities within sexes accounts for 29% (males) or 37% (females) of the remaining variation, so, as Figure 9 shows, more variation is among sites than between sexes. Because even SEX-DF varies much more among localities than between the sexes I have largely assumed that sexual dimorphism is a minor part of the total variation and pooled the sexes in many of the subsequent analyses.

Discrimination Among Localities

I computed a variety of DFs with the 18 MSFs and the metric and grid variables among the 27 localities from which I had 10 or more skins (the small samples from Gravelly Bay and West Minesing pooled with the nearby Tip of Long Point and East Minesing; Table 3), using the SPSS DISCRIMINANT programme.

Generally about half of the specimens were correctly identified to locality by 5 DFs, and 26 DFs did not increase this above 66%. Separate analyses of the sexes did not raise this much (54 & 57% for 5 DFs; the eigenvalues of the first 10 or so DFs were greater than that of SEX-DF), and division of the grid and metric data by SKIN SIZE to remove size differences did not depress the success of identification below 47% for 5 DFs. The first few DFs were similar in all analyses: about half of the discriminating variation was accounted for by the first DF, which was a SPOTTING factor, about 20% by the second, which was a GLANDS factor, and 5-7% by each of the next few DFs, which expressed combinations of size, LINEATION,

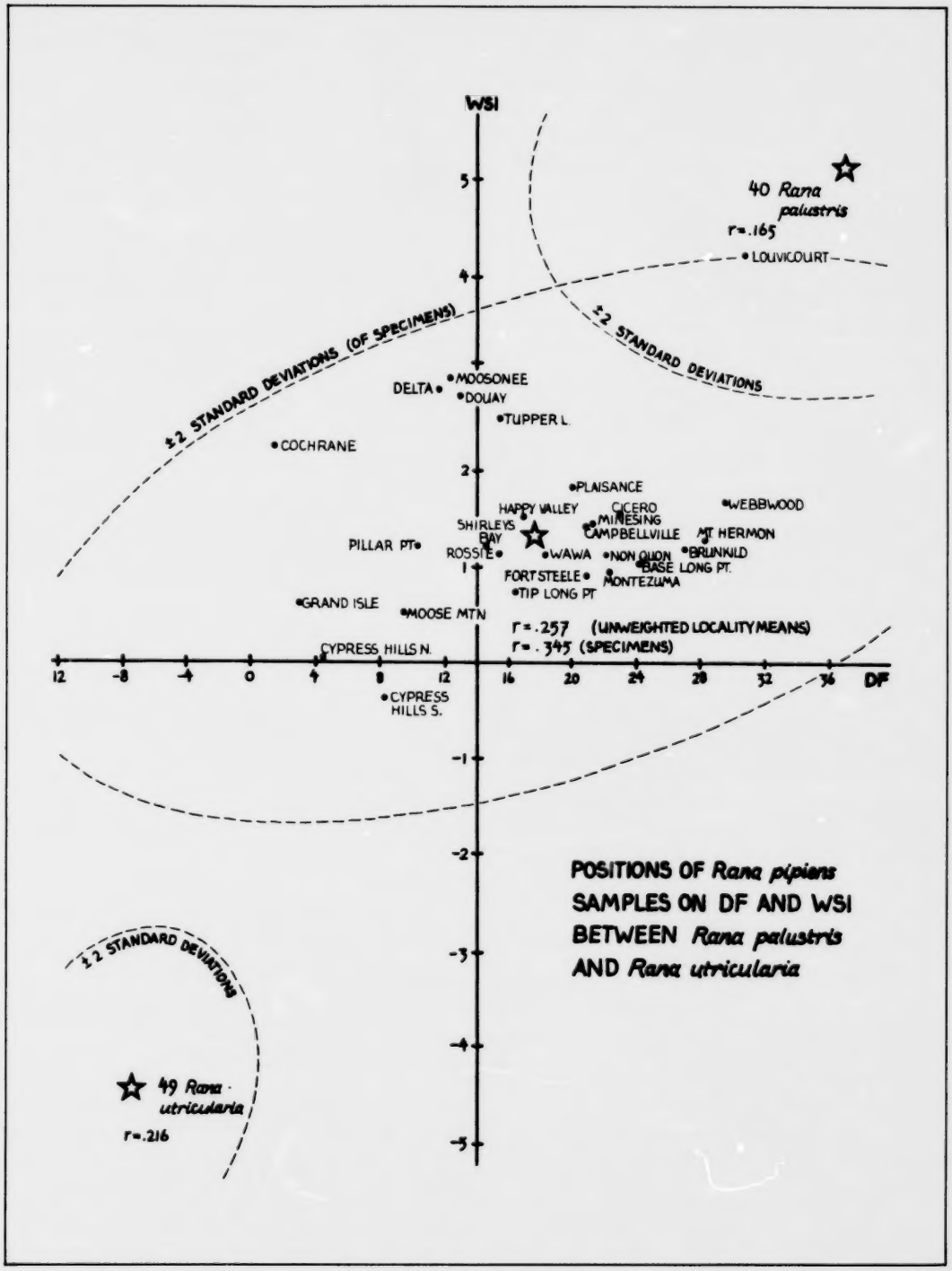

Figure 7. Locality means of the *Rana pipiens* samples on the *R. utricularia-palustris* discrimination variables.
This shows the position of the centroids of the samples of *Rana pipiens* (small dots) on the DF and WSI between *R. palustris* and *R. utricularia*, the centroids ± 2 s.d. for the specimens of the three species (large dots and ellipses), and the midpoints between *R. palustris* and *R. utricularia* (the position of the axes).

Table 7. Coefficients for WSI between *Rana utricularia* and *R. palustris*.

The score for a specimen is the sum of the coefficents of its character states. High values are *Rana palustris*-like; null entries are states not represented in the reference samples.

Character	Character States					
	0	1	2	3	4	5
NOSE SPOTS	-.45354	.91545	.95459	.95459	.95459	.95459
LEFT EYELID SP.	-.08163	.01721	-.18367	.18367		
RIGHT EYELID SP.	-.08163	.02336	-.22449			
NOSTRIL SPOT	-.04082	-.08185	.04686			
CANTHUS-ROSTRALIS LINE		.11636	-.02330	-.05900		
TYMPANUM SPOT	.25000	.30694	-.97959			
TYMPANUM OUTLINING		-.04082	-.10747	.13217	-.15447	
JAW MOTTLING	.00023	.01797	-.03082			
SPOT OUTLINING	-.11329	.11148	.27554			
DORSO-LATERAL FOLD COLORATION	.43277	-.26628	-.90459			
SPOT BILINEARITY	-.24018	-.14354	.56778			
LATERAL RETICULATION	.02928	.03011	-.23964			
LATERAL LINEATION	-.16550	-.05136	.53602	.63878		
FEMUR BACKGROUND	.43750	-.04114	-.87500			
FEMUR PATTERNING	-.00558	.01483	-.01612			
JAW GLANDS	-.43878	-.01448	.17946	.87959		
THROAT GLANDS	-.44898	.01936	.22793	.89796		
TIBIA GLANDS	-.39944	-.01228	.23000	.95000		
FEMUR PALENESS	.00043	-.04082				
DUSKINESS	.04383	-.12578				
LIGHT JAW LINE	.01244	.01145	.00314	-.13465		
JAW LINE INTENSITY	-.01020	-.03065	.03078			
DARK JAW LINE	-.01020	-.02020	-.00694	.06065	-.01872	-.03906

tibia spotting, and various of the MSFs. Since sex and size were responsible for little increase in the success of identification of specimens, sexual differences are confirmed as a minor element in the total variation, and size-related sampling accidents do not seem to contribute much to the differences between samples, so the DFs that I used as variables were computed from an analysis in which all the variables were used and in which the sexes were pooled.

The first DF (Figures 10&12) accounts for 48% of the total discriminating variance; 85% of its variation is among localities. Like the first MSF, it is a SPOTTING vector, and has by far its highest loading from that variable, which it contrasts with back area, so that specimens with high DF1 scores have little spotting and large back area. There are loadings in the same sense (*i.e.*, with the same sign) as SPOTTING for FEMUR LINE and TIBIA LINE, dorsal grid spotted (relative to unspotted), and tibia spotting, and FEMUR FULL BARS-MSF8, and in the opposite sense for LINEATION+MSF3.

DF2, with 19% of the total discrimination, contrasts dermal glands (VENTRAL GLANDS+MSF2, tibia glands, LONGEST GLAND LINE) with LINEATION+MSF3, and has some loading, in the sense of GLANDS, from SPOTTING+MSF1 and FEMUR LINE. Even when only VENTRAL GLANDS+MSF2, the tibia grid, and LONGEST GLAND LINE are used in DFA, GLANDS remains a single axis of variation. In this case it is the first DF (58% of the discriminating variance), SPOTTING (29% from the tibia grid) is second, and size (12%) is third.

DFs 3 and 4 contrast LINEATION and SPOTTING with dorsal and tibia spotting (Figures 11&12). In DF3, tibia and dorsal spot areas load in an opposite sense to the MSFs, and there are loadings in the sense of SPOTTING for light FEMUR BACKGROUND FEMUR LINE and FEMUR MIDLINE SPOTTING, and in the opposite sense for FEMUR DIAGONAL BARS, SPOT OUTLINING, and length of the longest dorsal spot.

DF4 contrasts SPOTTING, LINEATION, and tibia unspotted area with tibia spotted area and back size, though in this case dorsal and tibia spot area load in opposite senses. There is little contribution from other characters, though loadings from half bars on the femur and tibia and reduced FEMUR MIDLINE SPOTTING confirm that hind leg pigmentation is involved.

To see how these DFs are related to size, I created a variable from them which is uncorrelated with specimen size (DF3&4; the first PC

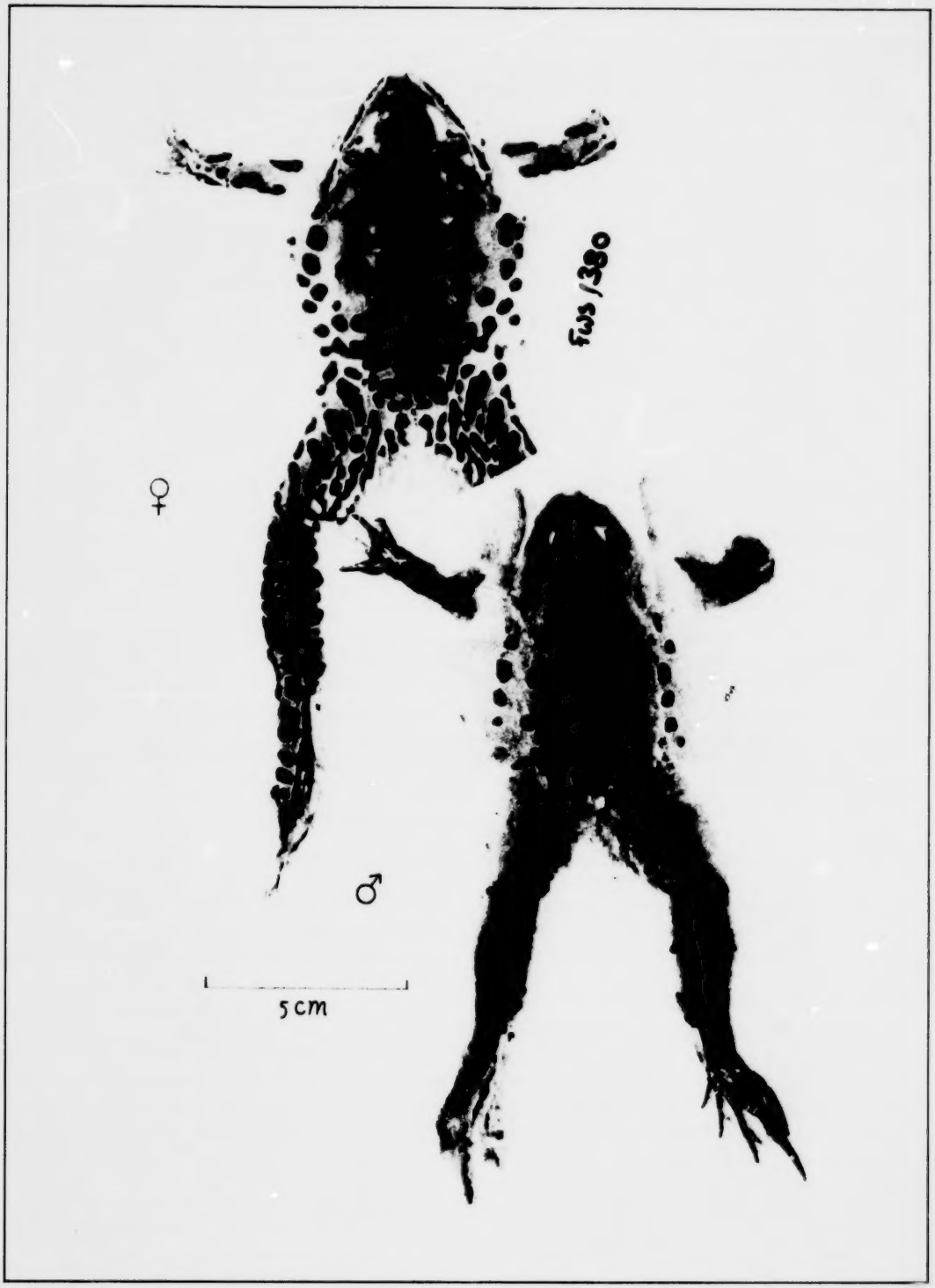

Figure 8. Skins with extreme values of SEX-DF.
♀ FWS 1380, from Rossie. SEX-DF=-2.78 ♂ FWS 4547, from Douay. SEX-DF=2.62

Table 8. Coefficients for DF between *Rana utricularia* and *R. palustris*.

This is not a standardized DF: the score for a specimen is the sum of the products of the raw data and the coefficients. High values are *Rana palustris*-like.

Character	Coefficient
NUMBER OF DORSAL SPOTS	-.32827
MERGED SPOT PRIMORDIA	0.12986
FEMUR FULL BARS	0.51683
FEMUR HALF BARS	-.18227
FEMUR BROKEN BARS	-1.31344
FEMUR DIAGONAL BARS	-.22217
TIBIA FULL BARS	3.76026
TIBIA HALF BARS	1.15002
TIBIA BROKEN BARS	1.39503
NOSE SPOT LENGTH	7.61470
SKIN LENGTH	-.10735
LONGEST GLAND LINE	0.23655
LONGEST DORSAL SPOT	-.13584
FEMUR MIDLINE	-.07134
FEMUR MIDLINE SPOTTING	-.50072
FEMUR LINE	-.11682
TIBIA LENGTH	-.15518
TIBIA LINE	0.24896

of the residuals of DF3 and DF4 from regressions of these DFs on SKIN SIZE). This PC accounts for 72% of the variation in the residuals and is correlated with the LINEATION-CC3 scores (p. 43 ff) at -.98 (Table 16), which confirms that DFs 3 and 4 are very nearly expressions only of size and a lineation-reduced-tibia-spotting factor (*e.g. CC3*) negatively and positively associated with body size.

DF5 has moderate loadings for a large number of characters superimposed on a back-tibia contrast like that in DF4. The ordering of dorsal and lateral spots in rows or lines is contrasted with LINEATION+MSF3, light tympanum spot, and merged dorsal spots.

Locality Variables

I did not make quantified records of the habitat or soil present at collecting sites, and I am not sure that I know what habitat variables should or could be measured (except for soil texture; Tracy, 1976) in studying geographic variation in *Rana*. I measured 11 characters (Table 10) on National Series topographic maps of the collecting localities. Dole (1971) found juvenile *R. pipiens* that had moved 5 km from their natal pond, so I evaluated the variables within a 5 km radius of the collecting site. While the topographic characters are doubtless reliable, the vegetation and wetland characters may suffer from inconsistancy in mapping and variation in the vegetation differences mapped. Differences among habitats are probably so great that errors of the first sort will be unimportant, while the variation in vegetation types should reflect climate, which is described by the climate variables. I used the base 10 logarithims of the measurements that have only an indirect connection with the collecting site (WETLAND SIZE, RIVER WIDTH, LAKE SIZE, HIGHER ELEVATION, LOWER ELEVATION) for the computations, since it seemed unlikely that the difference in size between, say, Nighthawk Lake and Lake Ontario would effect frogs in direct proportion to their areas. The data are listed in Table IX-2 of Schueler (1979).

There are four PCs of the correlation matrix (weighted by the number of specimens from each site) of these variables with eigenvalues greater than one variable. Together they account for 76% of the total variation. The first PC (30%) contrasts forested sites of high relief with those with extensive lakes and marshes, the second (19%) contrasts sites of high elevation with those with rivers and swamps, the third (16%) opposes FIELD AREA with FOREST AREA and all the other variables (except RIVER WIDTH), while the fourth (10%) associates elevations above the collecting site with RIVER WIDTH and contrasts this with swamps and elevations below the site.

Geographic Variables

These are the terms of the sixth order binomial expansion of the latitude and longitude of the collecting sites, from an origin of 40°N 120°W, a transformation that I used to keep the numerical magnitude of the higher powers within reasonable bounds. These variables are the terms of a TSA for the specimens. The simple spherical coordinates introduce little distortion because the collections were made within fairly narrow latitudinal limits (the standard deviation of latitude is about 3°, Table 3). I call the latitude variable 'N' (north), and the longitude variable 'E' (east).

Climate Variables

To provide standards for climatic comparison I ordinated Nearctic localities on the basis of 144 thermal, moisture, and wind variables using PCA. My purpose in this was to 1) see how

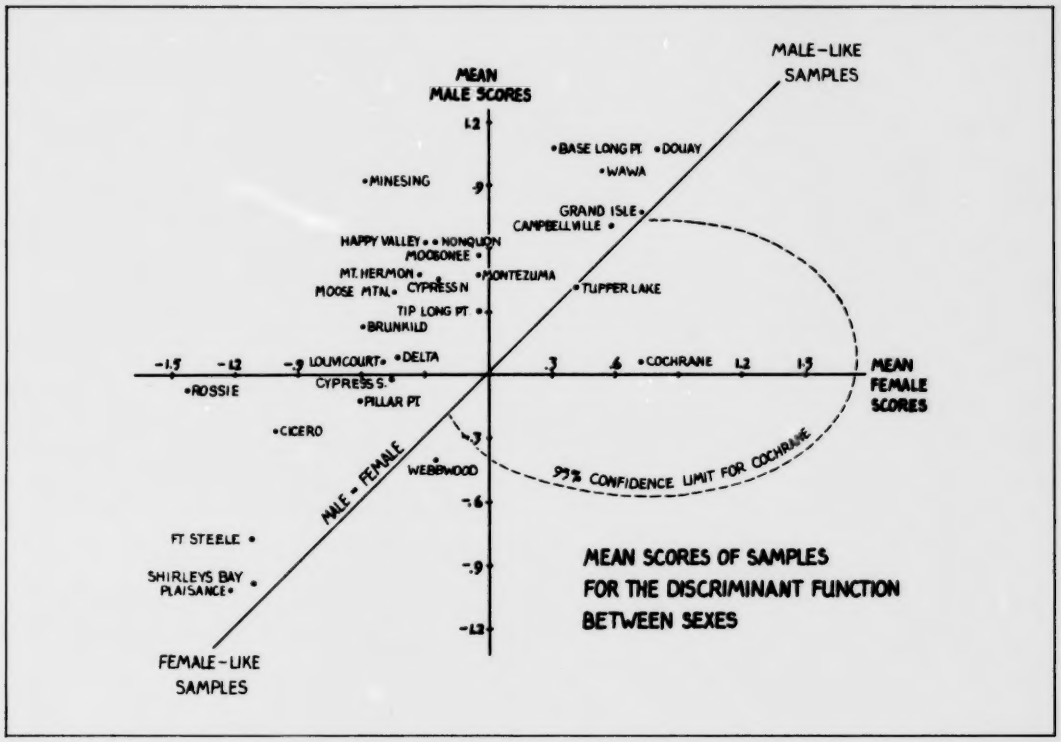

Figure 9. Mean scores of samples for SEX-DF.

This shows the position of the major samples ($n \geq 10$) on the DF between the sexes by plotting the mean score of the males in the sample on the vertical axis and the mean score of the females on the horizontal axis. High scores are male-like, so most points are above the male=female line. Only the small Cochrane sample (4 males, 8 females) is much below the line, so the 95% confidence intervals for its means are shown as an ellipse. Note that there is more variation among localities than between the sexes.

many composite characters are required to express much of the continental climatic variation, 2) provide variables which can conveniently be compared between studies, and 3) facilitate the use of these variables by providing equations for interpolations to localities other than the original stations.

I used the data tabled in Bryson and Hare (1974): 9 monthly means and 3 historical extremes of thermal, wind, and moisture variables (Table 11), except those with indirect or problematic biological impact (air pressure, wind direction) and composites of other variables (annual means, 18°C degree days; Blackith and Reyment, 1971). The localities were those in Bryson and Hare (1974) north of the Tropic of Cancer where sub-freezing temperatures had been recorded (Figure 13), totalling 124 stations. I excluded Mt. Washington, New Hampshire, from the analysis as it is a mountaintop site remote from the settled places where the other data originated and it accounted for 40% of the variance of PC3 (a wind and fog factor) when I included it. Mean vapour pressures for Alaskan stations were transformed from relative humidity, and for very cold months at northern Canadian stations were estimated as 80% relative humidity (Meteorological Branch, 1968 (4); List, 1971). The altitudes of Canadian sites are grossly in error in Bryson and Hare (1974) and I corrected them from Meteorological Branch (1968 (6)).

This matrix was too large for direct analysis by the programmes available to me, so I divided the variables into three groups of 48 by the type of observation they recorded (temperature, precipitation, and 'atmosphere' (wind, fog, clouds and humidity)), performed PCA (using NT-SYS) on the correlation matrix of each group, and then on the variance-covariance matrix of the PC scores (weighted by the square roots of the eigenvectors) of the PCs of as many components as were required to explain 98% of the variation in each group of the original variables. This procedure deleted 2% of the variation in the original

Table 9. Coefficients for SEX-DF.

Variables	Means		Standardized Coefficients*
	Male	Female	
1 SPOTTING	-.0317	0.0356	-.49593
2 VENTRAL GLANDS	-.0377	0.0366	0.02236
3 LINEATION	-.1082	0.1062	-.37089
4 HEAD SPOTS	-.0007	0.0003	-.01157
5 TIBIA BROKEN BARS	-.0816	0.0783	-.20925
6 FEMUR DIAGONAL BARS	-.0223	0.0265	-.11728
7 TIBIA HALF BARS	-.0321	0.0372	-.05594
8 FEMUR HALF BARS	-.0079	0.0135	-.04483
9 TYMPANUM SPOT	-.0461	0.0459	-.11818
10 SPOT OUTLINING	-.0705	0.0707	-.09550
11 NOSTRIL SPOT	-.0312	0.0308	-.08245
12 NOSE SPOT	0.0366	-.0369	-.09884
13 DUSKINESS	0.1083	-.1107	0.30639
14 FEMUR BROKEN BARS	0.0413	-.0380	0.00054
15 FEMUR BACKGROUND	0.0603	-.0597	0.06962
16 MERGED DORSAL SPOTS	-.0803	0.0835	-.24533
17 SPOT BILINEARITY	-.0063	0.0096	-.04329
18 LATERAL LINEATION	0.0521	-.0599	0.12827
	METRIC VARIABLES		
NOSE SPOT LENGTH	0.5162	0.5177	0.19395
LONGEST GLAND LINE	1.3575	1.3917	-.10673
LONGEST SPOT	1.0800	1.0975	0.13201
MIDFEMUR SPOTTING	1.1987	1.2364	-.23886
FEMUR LINE	0.8617	0.8527	0.23446
TIBIA LINE	1.2872	1.3155	0.01478
	GRID VARIABLES		
DORSAL SPOTTED	0.6746	0.6962	-.23467
DORSAL UNSPOTTED	0.8100	0.8311	-.63012
UNSPOTTED-NO GLANDS	0.7625	0.7774	0.09798
UNSPOTTED-GLANDS	0.6948	0.7209	-.10233
SPOTTED-NO GLANDS	0.7129	0.7278	-.07943
SPOTTED-GLANDS	0.6561	0.6852	0.13225

*High values are male-like.

variables, so that the secondary PCs are not exactly orthogonal relative to the original variables, but they are sufficiently uncorrelated to be useful expressions of unrelated climatic tendencies, and orthogonality is not preserved in the subset of localities involved in any single study.

I then computed the PC scores for the tropical Mexican stations, and first through sixth order trend surfaces for each of the first six PCs on a Sanson-Flamsteed projection of North America centred on the 100th meridian, using the SPSS Regression programme (Appendix 2). This projection collapses east-west distance onto a meridian (distance = longitude * cosine (latitude)) so that relative area is preserved and the parallels of latitude are straight lines perpendicular to the central meridian. Figures 15-20 are the maps from the analysis with the maximum 'F' and the sixth order map for each PC; the contour intervals are one standard deviation from the Nearctic mean of zero.

Eleven PCs were required to explain 98% of the variance in the temperature data, 22 for the precipitation data, and 16 for the atmosphere data. In the PCA of the station scores on these variables, the first 6 PCs accounted for 82% of the variance, which is 80% of the variation in the original matrix. Patterns of correlation with the original variables are shown in Figure 14.

WARMTH+PC1: This is a measure of the overall temperature at a locality, and is very strongly and uniformly correlated with all of the temperature variables (except daily temperature range), and with vapour pressure. It is more highly correlated with mean annual temperature than it is with any of the monthly variables

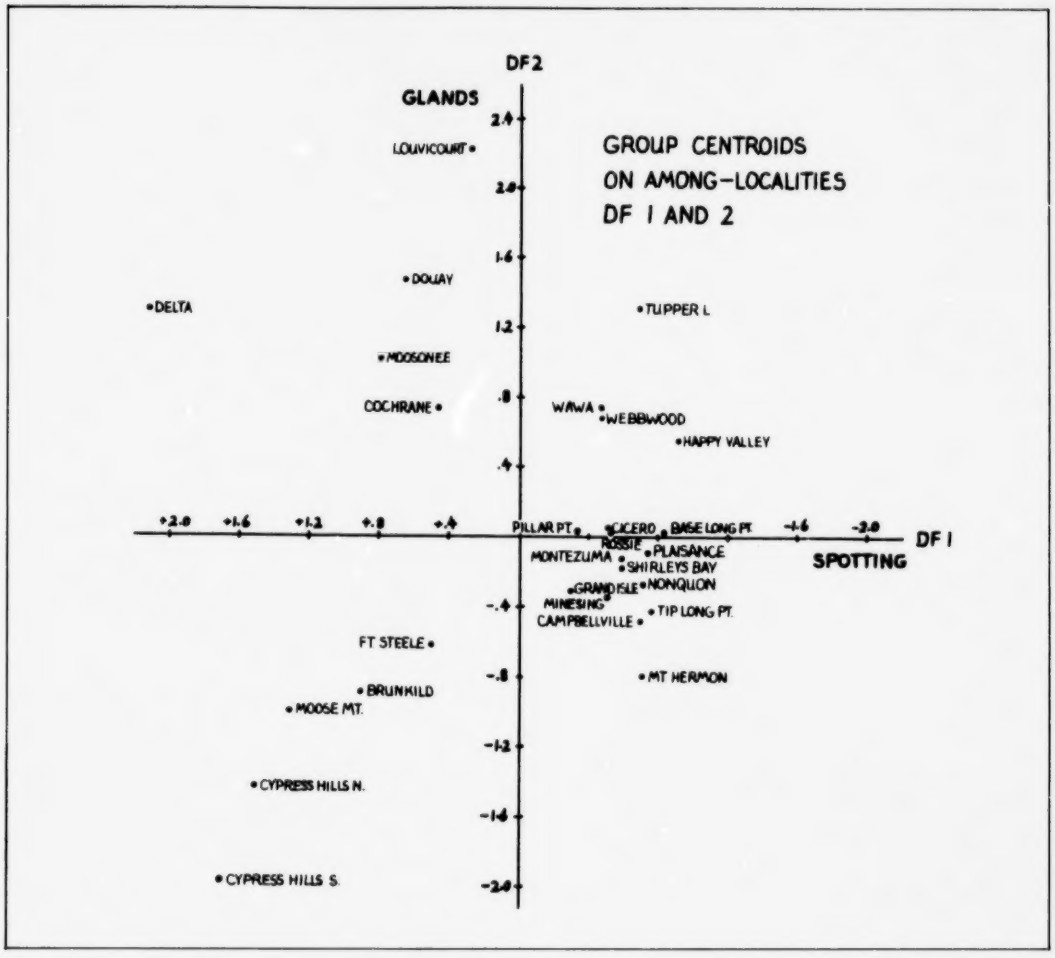

Figure 10. Centroids of samples on DFs 1&2.
In this plot high values of DF1 are to the left, so that SPOTTING (and east) is to the right, and GLANDS (and north) is up. Note that the western and northern samples are widely dispersed and that all of the samples from the lower Great Lakes drainage are tightly clustered together.

from which it was derived. WARMTH varies with latitude (r=-.92), and the linear TSA (with an F of 387) explains 86% of the variation in PC1. The cubic map is significantly better than the linear map, but the sixth order map is not significantly better than the cubic, though it does sketch in the details of variation in Mexico more clearly. Elevation explains a significant fraction of the remaining variation.

ARIDITY+PC2: Like PC1, PC2 is more strongly correlated with an annual variable than with any of the monthly characters on which it is based: in this case mean annual precipitation (r=-.88) ARIDITY+PC2 is strongly positively correlated with daily temperature range, and negatively correlated with all measures of water: cloudiness, days with fog, vapour pressure, and snow (except in the late summer and early fall), and is especially strongly correlated with mean, maximum, and days with precipitation. There is more seasonality expressed than in PC1: winter dryness is more strongly correlated with ARIDITY than summer dryness is, and there is a positive correlation with early snow. The stronger positive correlations with maximum temperature in the summer and stronger negative correlations with mean temperature in the winter are annual expressions of the greater temperature fluctuations in arid areas seen at the daily level in the correlations with daily temperature range.

Geographically ARIDITY is complex; it is the only PC which has a significant increment of variance explained in the sixth TSA map. This sixth order map only accounts for 73% of the variation, but precipitation is notoriously variable, even on a scale of metres (Yoshino, 1975), so there is probably a lot of 'real' error variance in the data.

Table 10. Locality characters.

All characters (except ELEVATION) are evaluated within a 5 km radius of the collection site on large scale national series topographic maps.

WETLAND SIZE: The base 10 logarithm of the product of the length and width of the largest continuous wetland, in square decametres.

RIVER WIDTH: The base 10 logarithm of the width of the widest stream, in metres.

LAKE SIZE: The base 10 logarithm of the product of the length and width of the largest body of water, in square decametres. When this was a lake in the course of a river RIVER WIDTH was measured at a point where there was some indication of sensible current, even if this was more than 5 km from the collecting site.

FOREST, FIELD, SWAMP, MARSH, & WATER AREA: The rank order, from 9= most extensive to 5= least extensive, 4= absent, of the area of these habitats, estimated from the extent of wetland symbols and green and blue overprinting on the topographic maps ('marsh' is white wetland, 'swamp' is green wetland). Apparent ties were given the average of the appropriate ranks, and in cases where one type was far more extensive than the next (> 80% of the remaining area) ranks were skipped.

ELEVATION: The elevation of the collecting site, in metres.

LOWER & HIGHER ELEVATION: The base 10 logarithm of the difference in elevation between the collecting site and the lowest and highest points (respectively), in metres.

SUMMER HUMIDITY+PC3: Of the 50 characters most strongly correlated with PC3 only one, maximum annual temperature (r=-.51), is an annual variable, and only wind speed (negative) and vapour pressure (positive) have the same sign relationship with PC3 throughout the year, so SUMMER HUMIDITY is a 'seasonality' factor analogous to the 'shape' factors of morphometrics. Stations with high scores on this PC are characterized by cloudiness, many days with precipitation, much precipitation, snow, fog, and little range of temperature from July to September, and the reverse conditions from December to May, with high humidity and low wind speed throughout the year. These conditions prevail in Mexico, Florida, and the Arctic, while low scores occur in the central part of the continent, especially in the Great Basin area. The cubic TSA shows this pattern, and has the highest F value, though the fourth order map, with a slightly lower F, explains significantly more of the variation.

WIND+PC4: This is strongly and uniformly correlated with wind speed throughout the year, and its relationships with other characters are quite seasonal. Stations with high WIND scores have winters with little snow or other precipitation, fog, or cloudiness, and low winter vapour pressure and temperature relative to the other seasons. The measurement of wind speed is even more site dependent than the measurement of precipitation, so the sixth order TS map of high winds in the southern Great Plains, around Hudson Bay, and in northwestern Alaska accounts for only 63% of the variation.

NO FOG +PC5: Stations with high scores for PC5 have little fog throughout the year, much precipitation, which is rain rather than snow, from May to September, and generally dry winters. Low scores, foggy, dry summers, occur along the coasts of California, Alaska, and Newfoundland, and in the eastern Arctic. There is a steady increase in the variation explained by the TSA, but the sixth order map explains only 60% of the variation.

FOG & SNOW+PC6: Fogginess is again a contributor to PC6, but in this case spring and summer fog is correlated with winter snow, summer precipitation, high daily range of temperature, and (r=.39) elevation. Even with two elevation terms the TS map explains less than half of the variation; it has high values in the Gulf coast of Florida and Mexico, the mountains of California, and the Maritime Provinces.

Estimating Climate Variables

I estimated each of the climate PCs at each collection locality in two ways, both of which present problems. The values I call the 'estimated climate variables' are predicted values for the sites from the sixth order TSAs of the PCs with two elevation terms (Appendix 2). The 'local climate' variables are the PCs calculated from the nearest available climatic data (data listed in Table IX-2 of Schueler, 1979; Meteorological Branch, 1968; U.S. Dept. of Commerce, 'Climates of the States'; a few data from U.S. Dept. of Commerce, 1967). Often 'nearest' was not very close, and different subsets of the variables were unavailable for some stations in the United States and Canada, so the values for many sites

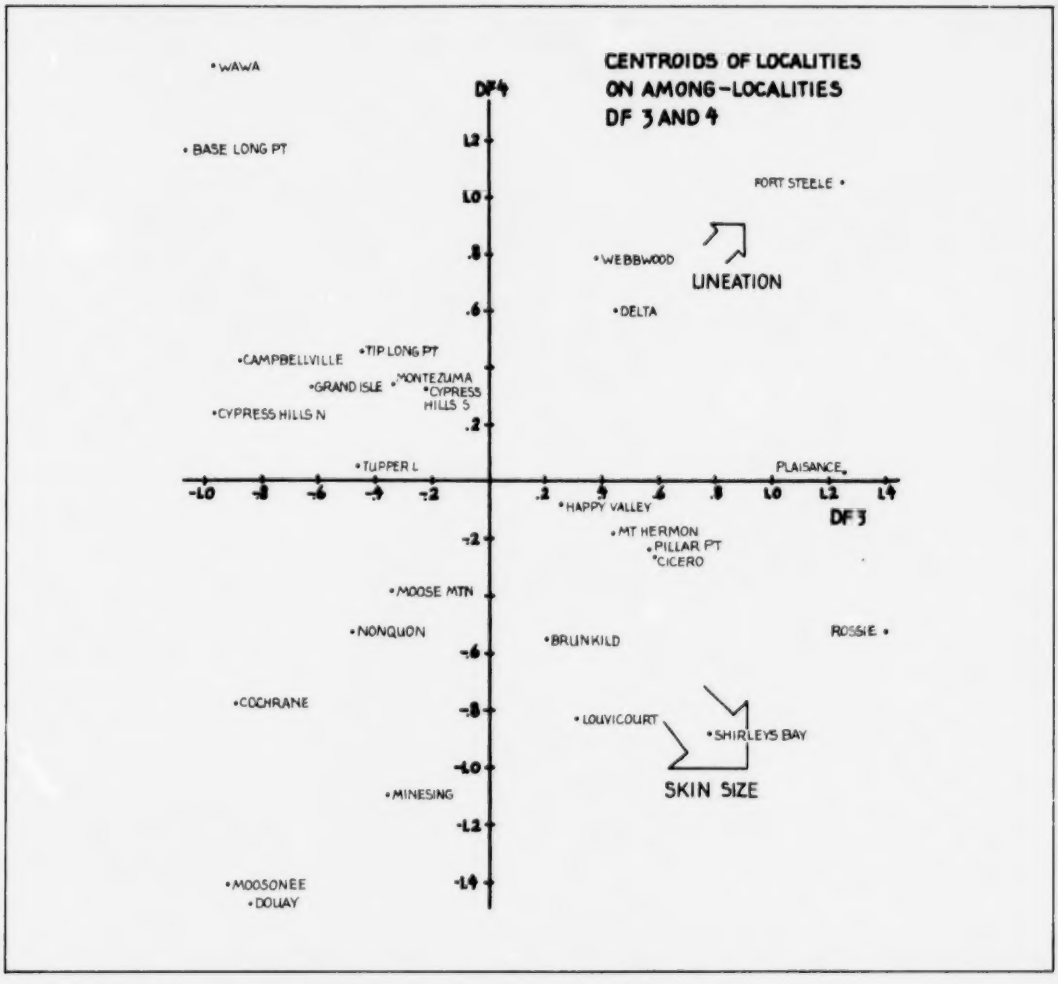

Figure 11. Centroids of samples on DFs 3&4.
The arrows indicating LINEATION and SKIN SIZE are based on the correlations of DF3 & 4 with SKIN SIZE and CC3. Note that the geographic ordering of localities is much weaker in this plot than in Figure 10.

are composites from several stations. A problem with using climatic data for the explanation of geographic variation is that the data are not gathered from the places where the specimens are collected. One solution to this problem, which studies of introduced species have used *de facto* (Johnston & Selander, 1971), is to study species that inhabit towns, airports, or other habitats where climatic data are recorded, and to collect the specimens at sites for which such data are available. The 'estimated' variables represent an alternative solution to this problem: to consider only that portion of the variation among stations which makes up a geographically simple pattern, such as that described by the TSA.

The correlation between the estimated and local variables are not very high (Table 12), and only WARMTH and ARIDITY clearly describe the same pattern in both variable sets.

Zoogeographic Variables

These variables are composites of the climate PCs based on the occurrence of species of *Rana* at the climate stations (Figure 13; Conant, 1975; Stebbins, 1966). I made them to help in the interpretation of patterns of geographic variation in terms of the zoogeographic relationships they express, so that, for example, if an attribute has the same pattern of geographic variation as the

number of species of *Rana* its variation can be thought of in terms of the overall favorableness of the climate for ranid frogs.

Computation was by the SPSS DISCRIMINANT and REGRESSION programmes. Values for the collecting localities were derived from the estimated climate variables. Correlations of the zoogeographic variables with some skin and climate and geographic variables are listed in Table 13.

Table 11. Climatic characters.

For each month:

TEMPERATURE (°C)
DAILY MEAN
MAIN DAILY RANGE
HISTORICAL MAXIMUM
HISTORICAL MINIMUM
PRECIPITATION
MEAN (mm of water)
HISTORICAL MAXIMUM IN 24 HOURS (mm of water)
MEAN SNOWFALL (cm = mm of water)
DAYS WITH PRECIPITATION (> 0.25 mm)
HUMIDITY, CLOUD, AND WIND
MEAN VAPOUR PRESSURE OF WATER (mbar)
DAYS WITH FOG
MEAN CLOUDINESS (tenths of sky)
MEAN WIND SPEED (m/sec)

Number of *Rana* as a Function of Climate (#RANA)

This is a regression of the number of species of *Rana* at the mainland climate stations east of the Cordillera on the climate variables and their quadratic combinations. A regression on ARIDITY alone explains 68% of the variation (F=141), and the inclusion of WARMTH**2 increases this to 78%, though the F declines to 125. The inclusion of more variables gives a further explanation of only 1% by each variable, so I have used the two-element equation, which is clearly interpretable: more species of *Rana* are found in areas of high precipitation and moderate temperature: #RANA=ARIDITY * -2.514688 + WARMTH**2 * -0.5724653 + 3.999594. The standardized coefficients are -.33717 and -.82283. Among the sites in the present study #RANA correlates with ARIDITY to the point of virtual identity (r=-.99; Table 13). Because the climate PC scores are standardized the constant term in the equation indicates that at a site with mean (0) Nearctic WARMTH and ARIDITY one finds four species of *Rana*.

Rana pipiens Range DF

This is a DF between 44 mainland stations east of the Cordillera where *R. pipiens* occurs and 41 where it does not (Figure 13), based on stepwise inclusion of the climate variables and their quadratic combinations. It is intended to serve as a measure of how peripheral to the range of the species the climate of a locality is. I used the quadratic terms because the range of a species which occupies intervals of climatic gradients cannot be discriminated by linear combinations of such gradients unless they appear in some curvilinear combination.

The inclusion of three variables, WARMTH, SUMMER HUMIDITY, and their product, gave the maximum F value (58), and resulted in a function which identified 95% of the stations correctly: WARMTH * -.91475 + SUMMER HUMIDITY * -.81614 + (WARMTH * SUMMER HUMIDITY) * 0.50212 - .10107 (the standardized coefficients are -.88535, -.59530, and 0.52906). This gives a plausible picture of the species' range: relative to the rest of the Nearctic *Rana pipiens* is found in cold areas with hot, dry summers, but the warmer parts of its range tend to have more humid summers. Among the localities of this study this variable is highly correlated with NO FOG+PC5 (r=0.81; Table 13).

DFs Among Climates Inhabited by Species of Leopard Frogs (Species-Climate DFs)

These are DFs among the climate stations within the ranges of *Rana pipiens* (51) *R. utricularia* (22), and *R. blairi* (7; Figure 13) based on the 6 climate variables. The first DF, with 94% of the discriminating variance, separates the range of *R. pipiens* from that of the other species on the basis of colder temperatures in the north, while the second separates *R. blairi* from *R. utricularia* on the basis wind, dry summers, cold, and aridity in the range of *R. blairi*.

Figure 12. Skins with extreme values of DFs.

These skins illustrate extreme (though not necessarily the most extreme) values of the first 4 DFs among localities. Since the DFs are standardized the values given below are standard deviations in the whole skin sample.

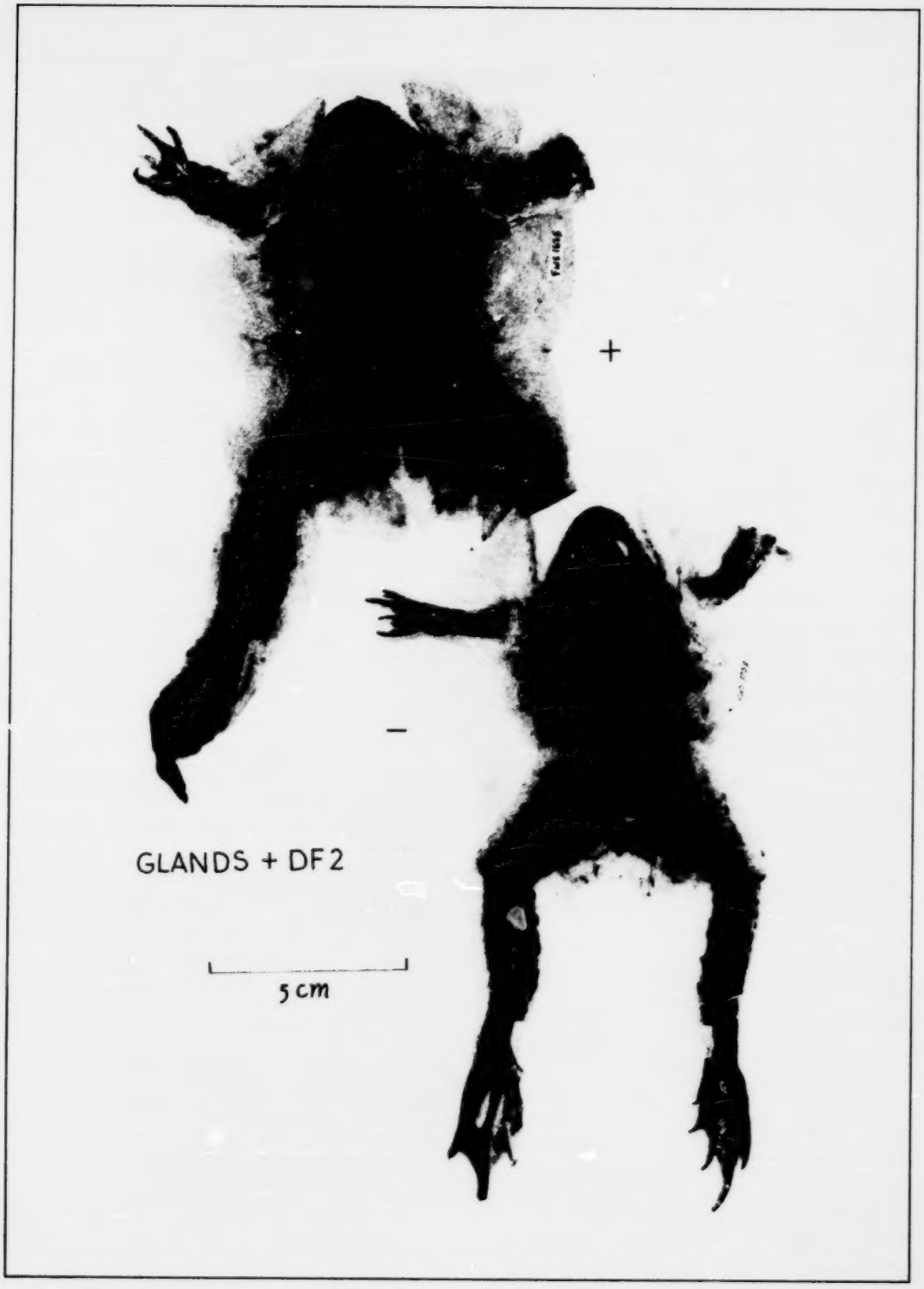

Figure 12. Skins with extreme values of DFs continued.

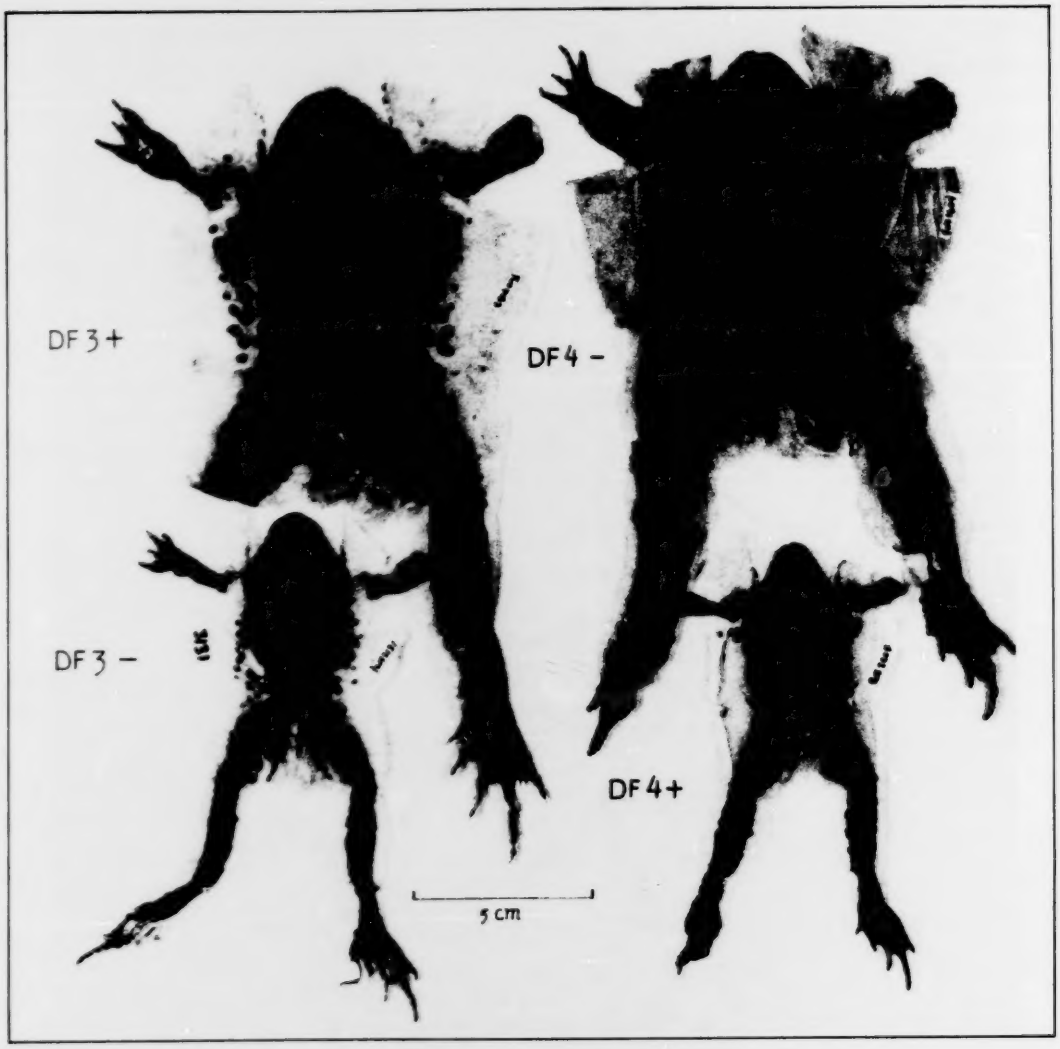

Figure 12. Skins with extreme values of DFs continued.

Specimen	Sex	Locality	Specimen Scores DF1	DF2	DF3	DF4
SPOTTING-DF1						
- FWS 2832	♀	E Minesing	-1.60	-.52	-.68	-.32
+ FWS 3925	♀	Delta	3.15	1.89	0.72	1.27
GLANDS+DF2						
+ FWS 1625	♂	Hoyle	0.91	3.04	-.42	-1.32
- FWS 3759	♀	Cypress Hills S	1.22	-2.65	-.09	-.04
SIZE(+)&LINEATION(+)DF3						
+ FWS 4037	♀	Plaisance	-1.25	0.40	3.17	-.85
- FWS 3131	♂	Nonguon River	-.45	-.40	-2.94	-.47
SIZE(-)&LINEATION(+)DF4						
+ FWS 3207	♀	Base Long Pt.	-.38	-.73	-1.14	3.12
- FWS 1624	♀	Hoyle	1.98	5.74	0.44	-3.32

Table 12. Correlations between 'estimated' and 'local' climate variables.

		LOCAL					
		WARMTH	ARIDITY	SUMMER HUMIDITY	WIND	NO FOG	FOG&SNOW
E	WARMTH	0.89	-.27	-.42	-.21	-.59	0.44
S	ARIDITY	-.41	0.86	0.04	0.44	-.13	0.06
T	SUMMER HUMIDITY	-.14	0.17	0.12	0.00	0.34	-.05
I	WIND	0.13	0.36	-.00	0.62	-.30	0.16
M	NO FOG	-.33	-.15	0.42	-.10	0.20	0.30
A	FOG & SNOW	-.57	-.07	0.15	-.13	0.39	0.41
T							
E							
D							

Correlations weighted by numbers of skins.

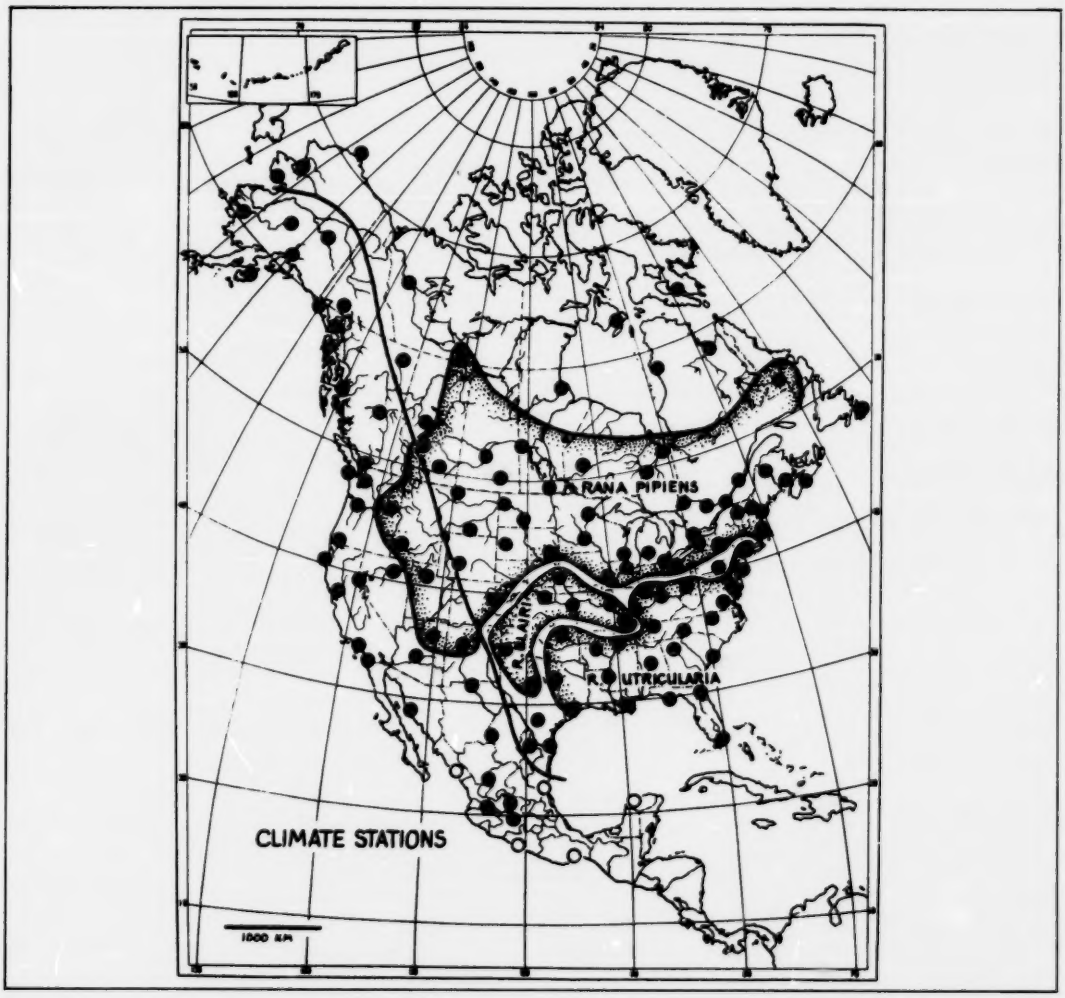

Figure 13. Climate stations.

Temperate (=Nearctic) stations are shown as dark circles, tropical stations as open circles. Mainland stations north and east of the heavy line were used in the regression of *Rana* species number on climate and in the *Rana pipiens* range DF.

Table 13. Correlations among zoogeographic variables and with estimated climate and some skin and geographic variables.

		#RANA	R. PIPIENS RANGE DF	SPECIES-CLIMATE DF1	SPECIES-CLIMATE DF2
R. PIPIENS RANGE DF		0.32			
SPECIES-CLIMATE	DF1	-.79	0.26		
	DF2	-.84	-.52	0.45	
WARMTH		0.51	-.53	-.92	-.17
ARIDITY		-.99	-.39	0.73	0.87
SUMMER HUMIDITY		-.03	0.03	0.30	-.10
WIND		-.61	-.41	0.37	0.82
NO FOG		0.08	0.81	0.35	-.28
FOG & SNOW		-.18	0.24	0.45	0.14
LATITUDE		-.80	0.16	0.96	0.48
LONGITUDE		-.91	-.35	0.68	0.69
SPOTTING+CC1*		0.88	0.22	-.78	-.72
GLANDS-CC2*		-.30	-.65	-.14	0.40
LINEATION-CC3*		-.06	0.19	0.16	0.05
SKIN SIZE		-.42	-.11	0.37	0.37

All correlations are weighted by the numbers of skins.

*See p. 43 ff.

Figure 14. Correlations of climate characters with PCs of climate.

This shows the correlations between the climate PCs as symbols which show the strength of the correlations scaled by the mean and standard deviations of the absolute values of the correlations of all the characters with each PC. The symbols are chosen to represent the magnitude of positive correlations by the number of intersecting lines in the field (+'s), and of negative correlations by the number of horizontal lines (-'s).

WARMTH+PC1

	JAN	FEB	MAR	APR	MAY	JUN	JUL	AUG	SEP	OCT	NOV	DEC
MNTEMP	######	######	######	######	######	######	######	######	######	######	######	######
RGTEMP	++	++	++	++	++				++	++	+++++	++
MXTEMP	######	######	######	######	+++++	+++++	+++++	+++++	+++++	######	######	######
MINTMP	######	######	######	######	######	######	######	######	######	######	+++++	######
MNPRCP				++	++	++						
MXPRCP	++	++	+++++	++	+++++	++	++	++	++	+++++	++	++
MNSNOW	--	--	--	-----	-----	--	--	--	-----	-----	-----	-----
DAYSPR	--	--	--	--	--	--	--	--	--	-----	-----	--
VAPPRS	######	######	######	######	+++++	+++++	+++++	+++++	+++++	######	######	######
FOGDAY					--	--	--	--	--			
CLOUDS				--	-----	-----	--	-----	-----	-----	-----	--
WINDSP	--			--	--	--	--	--	--	--	--	--

ARIDITY+PC2

	JAN	FEB	MAR	APR	MAY	JUN	JUL	AUG	SEP	OCT	NOV	DEC
MNTEMP	--										--	--
RGTEMP	######	######	######	######	######	######	######	######	+++++	+++++	######	######
MXTEMP					++	++	++	++	++			
MINTMP	--	--	--	--	--	--		--	--	--	--	--
MNPRCP	=====	=====	=====	=====	=====	-----	-----	-----	=====	=====	=====	=====
MXPRCP	=====	=====	-----	-----	-----	--	-----	--	-----	-----	=====	=====
MNSNOW	--	-----	--						++	++		--
DAYSPR	=====	=====	=====	=====	=====	------	--	--	-----	=====	=====	=====
VAPPRS	--	--	--	--	-----	-----	--	-----	-----	-----	-----	--
FOGDAY	--	--	-----	-----	-----	--	-----	-----	-----	-----	--	--
CLOUDS	------	-----	-----	-----	-----	-----	-----	-----	--	--	-----	-----
WINDSP	-----	-----	--	--						--	-----	-----

SUMMER HUMIDITY+PC3

	JAN	FEB	MAR	APR	MAY	JUN	JUL	AUG	SEP	OCT	NOV	DEC
MNTEMP	++	++				--	-----	--	--			++
RGTEMP			++	--	-----	=====	=====	=====	=====	=====	-----	
MXTEMP			--	-----	=====	=====	=====	=====	-----	--		
MINTMP	++	++	++			++	++	++	+++++	++	++	++
MNPRCP	--	--	-----	-----	-----		+++++	+++++	+++++		--	--
MXPRCP	--	--			-----				++	++		--
MNSNOW	-----	-----	-----	--		++	+++++	+++++	+++++	++	--	-----
DAYSPR	=====	=====	=====	=====	-----		#####	#####	#####	++	--	-----
VAPPRS	+++++	+++++	++	++	++	++	++	++	+++++	+++++	+++++	+++++
FOGDAY	--	-----	-----			++	++	++	++	++	--	--
CLOUDS	=====	=====	=====	=====	--	+++++	#####	#####	#####	++	-----	-----
WINDSP	-----	-----	-----	-----	-----	-----	--	--	--	--	--	-----

WIND+PC4

	JAN	FEB	MAR	APR	MAY	JUN	JUL	AUG	SEP	OCT	NOV	DEC
MNTEMP	--	--	--	--	++	++	++	++	++	++	--	--
RGTEMP	++	++	--	--	--	--	--	--	--	++	++	++
MXTEMP		--	++	++	++	++	++	+++++	++	++	++	++
MINTMP	--	--	-----	--	--	++	++	++	--	--	--	--
MNPRCP	-----	-----	--	++	+++++	+++++	+++++	+++++	++	--	-----	-----
MXPRCP	-----	--	++	+++++	+++++	#####	#####	#####	+++++	+++++	--	-----
MNSNOW	--	--	--	--	++	++	++	++	++	++	--	--
DAYSPR	-----	-----	-----	--	++	++	++	++	++	--	-----	-----
VAPPRS	--	--	--	++	++	+++++	+++++	+++++	++	++	--	--
FOGDAY	-----	-----	--	++	++	++	++	--	-----	-----	=====	=====
CLOUDS	-----	-----	--	--	++	++	++	++	++	--	--	-----
WINDSP	#####	#####	#####	#####	#####	#####	#####	#####	#####	#####	#####	#####

NO FOG+PC5

	JAN	FEB	MAR	APR	MAY	JUN	JUL	AUG	SEP	OCT	NOV	DEC
MNTEMP	--	--			++	++	++	++			--	--
RGTEMP	+++++	+++++	+++++	+++++	+++++	+++++	++	++	++	++	++	++
MXTEMP				++	++	++	++	++				
MINTMP	-----	-----	-----	--	--			--	--	--	-----	-----
MNPRCP	--	--		++	#####	#####	#####	#####	#####	+++++	++	--
MXPRCP	--	--	++	++	+++++	+++++	+++++	+++++	++			--
MNSNOW	++	++			--	--	-----	-----	--	++	++	++
DAYSPR	++	++	++	++	#####	#####	#####	#####	#####	+++++	++	++
VAPPRS	--	--			++	+++++	+++++	++	++		--	--
FOGDAY	=====	=====	=====	=====	=====	=====	=====	-----	-----	=====	=====	=====
CLOUDS	++	++	++	++	++	+++++	+++++	+++++	+++++	++	++	+++++
WINDSP	-----	-----	-----	-----	-----	=====	=====	=====	-----	-----	-----	-----

FOG & SNOW+PC6

	JAN	FEB	MAR	APR	MAY	JUN	JUL	AUG	SEP	OCT	NOV	DEC
MNTEMP	++	++	++	++	++	++	++	++	++	++	++	++
RGTEMP	#####	#####	#####	+++++	#####	+++++	+++++	+++++	+++++	+++++	+++++	#####
MXTEMP	++	++	++	++	++	--	--	--	--	++	++	++
MINTMP	++	++	++	++	++	--	--	--	++	++	++	++
MNPRCP	++	++	--	++	++	+++++	#####	#####	#####	+++++	++	++
MXPRCP	--	--	-----	--	--	--	++	++	+++++	--	--	++
MNSNOW	#####	#####	#####	#####	+++++	++	-----	-----	--	--	+++++	#####
DAYSPR	--	--	-----	--	++	+++++	#####	+++++	+++++	++	--	--
VAPPRS	--	--	--	--	--	-----	--	--	--	++	++	--
FOGDAY	+++++	#####	#####	#####	#####	#####	#####	#####	#####	+++++	+++++	++
CLOUDS	-----	=====	=====	-----	-----	--	++	--	--	--	-----	=====
WINDSP	++	++	++	++	++	++	--	--	++	++	--	--

Correlation		Range of absolute values
positive	negative	
#####	=====	r > (mean + 1 s.d.)
+++++	-----	(mean + 1 s.d.) > r > mean
++	--	mean > r > (mean - 1 s.d.)
null	null	(mean - 1 s.d.) > r

Full character names are given in Table 11 in the same sequence.

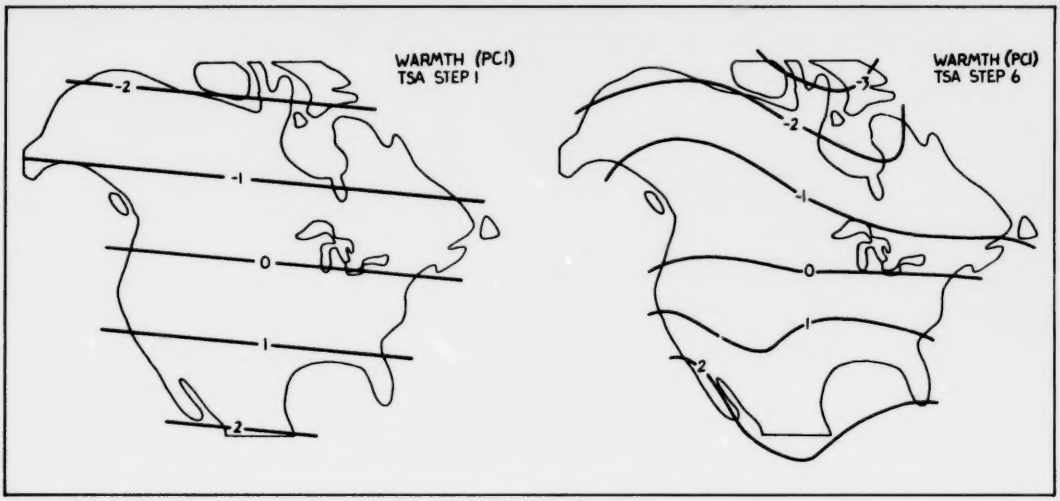

Figure 15. TSA of WARMTH+PC1.

Contour maps of the Trend Surface with the maximum F (linear, F=387, 86% of variation explained), and the 6th order surface (F=69, 93% explained). WARMTH is the most simply patterned of the climate variables; the linear surface has a north-south pattern that is slightly modified in subsequent steps, as the cooling effects of various water bodies become evident.

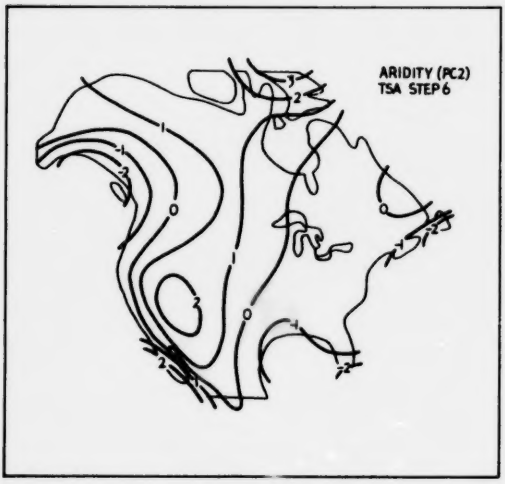

Figure 16. TSA of ARIDITY+PC2.

Contour map of the 6th order Trend Surface, which has the maximum F value (F=18, 73% of variation explained). ARIDITY shows a geographic complexity which is reminiscent of the large number of PCs required to extract 98% of the variation in the precipitation data: the F value continues to increase through the 6 steps and with the elevation terms. The pattern which emerges is familiar; the North Pacific and Atlantic coasts are humid and the interior, especially the south west and the west coast of Mexico, and the eastern arctic are arid.

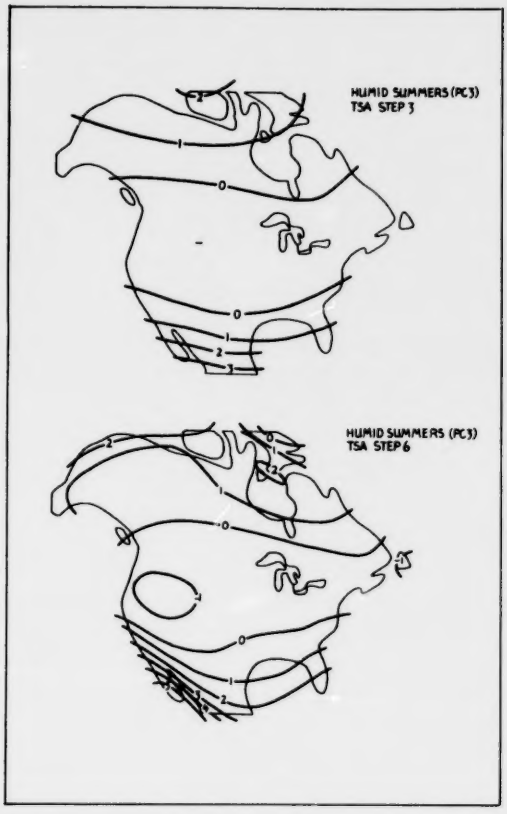

Figure 17. TSA of SUMMER HUMIDITY+PC3.

Contour maps of the Trend Surface with the maximum F (cubic, F=52, 78% of variation explained), and the 6th order surface (F=35, 88% explained). Relatively dry summers and wet winters are characteristic of the mid-latitudes of the Nearctic, especially the Cordillera. This is basically a quadratic pattern, though the cubic and fourth order maps fill in the details and represent significant increases in variation explained.

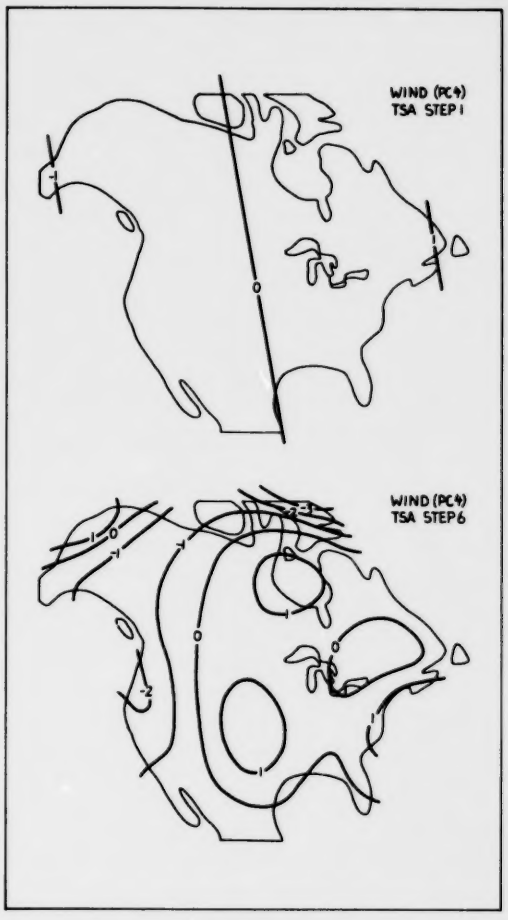

Figure 18. TSA of WIND+PC4.

Contour maps of the Trend Surface with the maximum F (linear, F=16, 19% of variation explained), and the 6th order surface (F=9, 64% explained). Only 65% of the variation in WIND is explained by the total TSA, which is doubtless due to the extreme micrometeorological variation in the measurement of the strength of the wind. While there is a gradual increase in the variation explained, the sixth step map is perhaps the most instructive: it shows windy areas in the southern Great Plains, west of Hudsons Bay, and along the central Atlantic coast and the north slope of Alaska, and calm areas in the eastern arctic and the central Pacific coast.

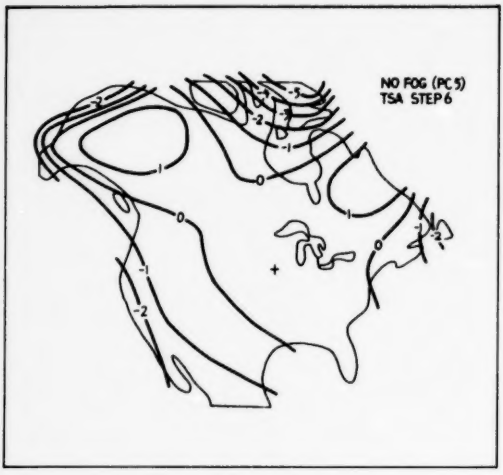

Figure 19. TSA of NO FOG+PC5.

Contour map of the 6th order Trend Surface, which has the maximum F value (F=8, 60% of variation explained). The steady increase in F values gradually resolves the Trend Surface into the simple pattern that coastal areas, except in the south east, are foggy. Two artifacts may be distorting the northern part of the map, however: there are no data for coastal Labrador, and the criterion (extent of visibility) for a 'foggy day' in Alaska was foggier than in Canada and the contiguous USA.

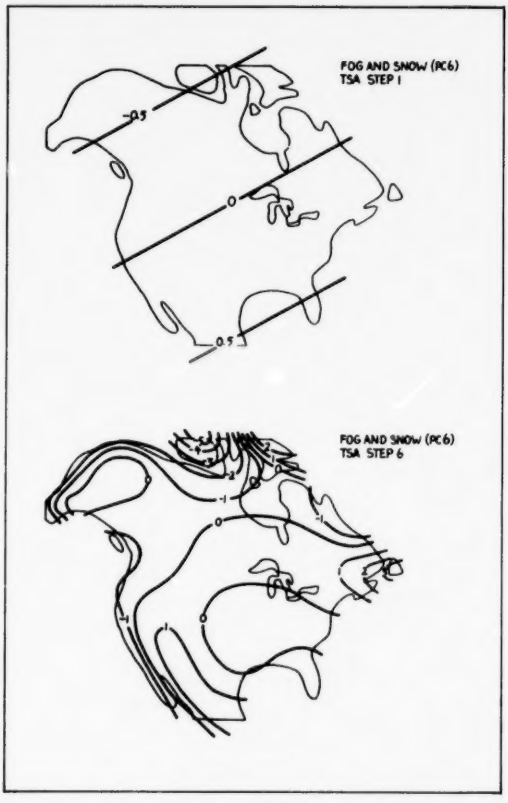

Figure 20. TSA of FOG & SNOW+PC6.

Contour maps of the Trend Surface with the maximum F (linear, F=6, 7% of variation explained), and the 6th order surface (F=4, 39% explained). Variation in FOG & SNOW seems to be very local and less than half of it is accounted for by the analysis, which gradually works out a pattern of irregularly coastal values, high in the east and low in the west. The extreme arctic values are plotted in areas from which there are no data.

Part III: Variation

Introduction

This Part treats four questions: (1) how much of the variation in the data is among rather than within local samples, (2) whether the variation among localities represents the same covarying characters as vary within local samples, (3) how much of the variation among localities is arranged in relatively simple geographic patterns, and (4) whether plausibly causal environmental variables can be found to account for more of the variation than purely geographic ones do.

Univariate Variation Among Localities

I assessed variation among localities by ANOVA with the specimens in the 27 groups used for the DFA. All of the variables except HEAD SPOTS and FEMUR BROKEN BARS have significant interlocality variation in at least one sex (Table III-1 Schueler, 1979). The proportion of variation among groups is roughly equal between the sexes, but is generally somewhat higher for females, though not often significantly so. Much of this difference, which is most marked in the size variables, is likely due to the fact that females have a greater size range than males, and that this size range was variously subsampled at each collection site both by natural events (Dole, 1965) and by my collecting techniques.

In the multivariate composite character sets (MSFs, DFs, and PCAs of sample means and of specimens) the first axis is the most variable among samples (85-61%) and there is a gradual falling off to about 15-25% after about the fourth axis. Most of the *a priori* indices and metric and grid variables have about half to a quarter of their variation among groups; SPOTTED NO GLANDS, RETICULATION INDEX, and GLAND INDEX are the most variable among groups.

Patterns of Variation Within and Among Samples

Multivariate studies of highly organized character sets have often found that the same characters covary in within- and among-population analyses (Johnston, 1973, *Passer* sparrows; Bryant, 1977, and Bryant & Turner, 1978, muscid flies; Sokal & Thomas, 1965, *Pemphius* aphids; Calhoon & Jameson, 1970, and Vogt & Jameson, 1970, *Hyla regilla*). Such covariation, especially if temporal and geographic trends are correlated with the same environmental variables, suggests that there is a relatively simple genetic or developmental basis for the variation. Such correlations may describe a selective-environmental axis with which the lineage has had long experience, so that gene complexes which promote variation along the axis have been favoured (Johnston, 1973) by Sysiphean selection (Williams, 1975). If, for example, small frogs are favoured when there is abundant rainfall and backgrounds are green, and large frogs are favoured when there is little rainfall and backgrounds are brown, then in an unpredictable climate the supression, genetic or developmental, of small brown and large green offspring will be selectively favoured, since these types will be unlikely to succeed in any weather (adaptive differences from Nevo, 1973 a&b).

Kluge and Kerfoot (1973) presented analyses of several data sets which supported their hypothesis that the within and among population variability of attributes should be correlated, since the among-population variation is an evolutionary extension of within-population variability. Sokal (1976) and Baker (1980) suggested ways of compensating for confounding size effects, but because of the arbitrary nature of the multistate character states I have plotted the within and among sample standard deviations of the multistate characters and metric and grid variables (Figure 21; the methods of Sokal (1976) and Baker (1980) give much the same results). The Kluge-Kerfoot effect is markedly evident both in this plot ($r=0.82$, $p<<0.001$; Olmstead-Tukey Corner Test) and in plots of single sets of variables. The attributes that show marked among-population variation are the multistate glands characters, lateral head pattern elements, counts of spots on the back and full and half bars on the legs, and the length of the FEMUR and TIBIA LINEs. Head spotting, SPOT OUTLINE, and infrequent femur barring characters had the least within-sample variation (Figure 21).

In a PCA of the pooled within-groups correlation matrix, the first PC represented size associated with increased gland area, the second PC spotting, and the third glands independent of size (Schueler, 1979).

Variation in Variability

To look for geographic or habitat patterns in the variability of the metric and grid variables I computed a PCA of the standard deviations of these variables for samples of 10 or more skins (sexes pooled) based on correlations weighted by the number of specimens.

The first PC describes variation in specimen size, while the subsequent ones pick out patterns of variability unique to one or two characters or localities (Figure 22). PC1 is correlated with the standard deviation of SKIN SIZE at 0.64 and has high loadings from all of the variables except the TIBIA and FEMUR LINEs, NOSESPOT LENGTH and the tibia no-glands characters, so it mostly expresses size variability, perhaps associated with variability of gland coverage. So much of this variability is related to the habitat and other circumstances of my collecting that no adaptive interpretation can be placed on this factor. PC2 and PC3 define outlying samples with peculiar patterns of variation. Delta and Tip of Long Point, respectively, have values at least a standard deviation above any other samples. Delta frogs have high variability in the tibia no-glands grid variables and TIBIA LINE, and Tip of Long Point has many little-spotted and some (dorsally) unspotted frogs, so that PC3 has high loadings from variability of dorsal spotting, LONGEST SPOT, NOSESPOT LENGTH, and FEMUR LINE. PC4 has high loadings only from these two latter characters, but contrasts them. PC5 (7.8% of the total variation) contrasts high variability of LONGEST SPOT at Shirleys Bay and the Cypress Hills with the other localities, PC6 (5.9%) separates Campbellville from the other localities by a standard deviation, and PC7 (5.2%) contrasts Shirleys Bay and Wawa with Base of Long Point and Cypress Hills South.

Since there is no PC before the seventh that is not dominated by a single attribute (counting size as one attribute) or a single locality, I conclude that there is no evidence of patterns of variability, and that pursuit of environmental correlates of the PCs would be unwarranted. It is worth noting, however, that PC2 and PC3 represent the reduction in mean SPOTTING (Delta) and SPOT AREA (Delta and Long Pt.) to regionally extreme low values in the only samples collected among sand dunes in *Typha* marsh. This habitat may well reduce the value of disruptive coloration (it favours energy coloration over striping in *Thamnophis sirtalis* at Long Point (Gibson, 1978) and unspotted frogs in *Rana utricularia* (Brown & Funk, 1977)), so the distinctness of PC2 and PC3 may argue for different increases in pattern variability in similar habitats by eastern and western *Rana pipiens*.

Patterns of Geographic Variation

To describe the patterns of geographic variation in the variation among localities, I computed CCAs between the skin variables (MSFs, metric and grid variables) and the geographic variables, entered by exponential order as in TSA. In each analysis (linear, quadratic, cubic) this produced trend surfaces which I compared with composite skin variables to see how similar the among-localities variation in each was to the generalized patterns.

Three geographic patterns appeared in all of the analyses ($r>.80$, weighted by specimens, no others had $r>.55$). These represent the previously identified axes of SPOTTING, GLANDS, and LINEATION (Figures 23,24). The coefficients of the patterns are listed in Appendix 2. The geographic patterns are nearly as effective as the DFA in explaining the among-site variation on these first three axes though they employ only 2, 5, and 9, rather than 26, independent variables (Figure 23).

Geographic Pattern 1: The dominant pattern of variation in pigmentation is this southeastward increase in SPOTTING (Figure 25, Pattern 1). The linear surface accounts for 68% of the variation in SPOTTING-DF1. Quadratic and cubic refinements of the linear pattern, which differ from it mostly in flattening the surface around the Great Lakes, raise this to 74 and 78%. This pattern is correlated with SPOTTING-DF1 at -.82 (linear) to -.88 (cubic), with SPOT AREA at 0.52 to 0.61, and with SPOTTING+MSF1 at 0.72 to 0.79. The other MSF and *a priori* composite variables with highest correlations ($>.10$, ($p>.01$)) with this pattern, and their correlations with the cubic pattern are: RETICULATION INDEX (.71), UNIFORMITY INDEX (-.53), sums of femur and tibia spotting (.29, .27), FEMUR HALF BARS MSF+8 (-.19), MERGED DORSAL SPOTS MSF+16 (.16), *UTRICULARIA-PALUSTRIS* DF (-.16), LATERAL LINEATION MSF+18 (.12), TIBIA HALF BARS MSF+7 (-.11), and SKIN SIZE (-.33); the NUMBER OF DORSAL SPOTS is correlated with the linear pattern at -.25 and with the cubic pattern at -.20. Environmental

variables with r>.40 with cubic Geographic Pattern 1 are ELEVATION (-.62), SWAMP AREA (.49), and HIGHER ELEVATION (.42), and estimated ARIDITY (-.89), WIND (-.73), and WARMTH (.61).

Geographic Pattern 2: This pattern of high values in the northeast (Figure 25, Pattern 2) is correlated only with the dermal gland variables. The linear pattern accounts for 31.6% of the variation in GLANDS+DF2, little more (32.7%) as the quadratic pattern, but 48.9% as the cubic pattern; this is about 70% of the among-sites variation, but much of the increase in variance explained by the cubic pattern depends on the fortuitous north-south alignment of the few western samples, which have extreme values for the GLANDS variables (compare Figure 25 with Figure 31).

The correlations of the dermal glands variables with the quadratic pattern are quite uniform: .50 (MSF2) .44 (GLAND AREA), .53 (GLANDS INDEX), .39 (*UTRICULARIA-PALUSTRIS* WSI), and .57 (DF2). Environmental variables with r > .35 with quadratic Geographic Pattern 2 are ELEVATION (-.36) and FIELD AREA (-.36) and estimated NO FOG (.75), WARMTH (-.70), SUMMER HUMIDITY (.53), and FOG & SNOW (.49).

Geographic Pattern 3. This is a saddle-shaped pattern of low values in southern Ontario and central New York and in the boreal forest of northwestern Quebec and northeastern Ontario and of high values in an eastern area centred on the Ottawa valley and a northwestern area centred on the Fort Steele sample (Figure 25, Pattern 3). Among the skin variables this pattern is most highly correlated with DF3, LINEATION and small size, (0.39 quadratic, 0.48 cubic) and LINEATION-CC3 (see beyond, -.26 and -.40); it is positively correlated with SKIN SIZE and negatively correlated with SEX-DF (in which size and lineation are positively associated). DARK FEMUR BACKGROUND+MSF15 has its highest correlations with this pattern (-.21), and LINEATION+MSF3 has one of its strongest correlations with cubic Pattern 3 (0.28), though it has equal correlations with Pattern 2 (-.28, -.30, -.23).

Cubic Pattern 3 is correlated above .23 with only four environmental variables: estimated NO FOG (-.54), HIGHER ELEVATION (0.44), RIVER WIDTH (0.37), and FIELD AREA (0.34).

Geographic patterns beyond the third have low eigenvalues (Figure 23), and their strongest correlations are with DF5 (Cubic Pattern 4, 0.26), DF4 (Cubic Pattern 4, 0.23), and NUMBER OF DORSAL SPOTS (Cubic Pattern 4, 0.23; Figure 24).

Canonical Correlations Between Skin and Environmental Variables

I computed CCAs between the metric, grid, and MSF variables and five combinations of the environmental variables for each sex and for all of the specimens together (SPSS programme CANCORR; Tables 14&15). For the first three CC axes the results were quite consistent through all of the analyses in that each had a heavy loading from its respective MSF, and in the case of the analysis for which I calculated the CC scores there was a remarkably close relationship with the first four DFs among localities and with the geographic patterns (Table 16). This suggests that the skin data are so much more structured than the environmental data that these CCAs (and the geographic patterns) should be thought of more as regressions of the kinds of skin variation on the environmental variables than as the extraction of kinds of variation common to the two variable sets but not dominant in either.

In the CCs between the skin variables and the separate sets of environmental variables the first CC has 70-80% of the variation in common between the two variable sets, the second 40-60%, and the third 20-40%. Estimated climate gives the best fit with SPOTTING (79%), and the locality variables give the best fit with GLANDS (50%); the two are about equal for LINEATION (29-30%).

As in the case of the ANOVAS the differences between the patterns of variation of the sexes seem to support the hypothesis that the pigmentation of females is more sensitive to the environmental variation than that of the males is (female CCs are stronger 18 times in Table 14, and weaker 5 times). If patterns of visual predation are such that females respond more sensitively to the frogs' terrestrial habitat, then in a geographically variable character the females should follow topographic and habitat variation more closely than the males do; there should be no difference between the sexes in the variability of dermal glands, and females should vary more than males in a variable mostly determined by habitat type. Given such a characterization of

each of the first three CC axes (SPOTTING, GLANDS, and LINEATION as described below: this is not an *a priori* prediction) the data fits this pattern. For SPOTTING the females have the closer relationship with the environmental variables only for the locality variables, there is no difference in GLANDS, and the females track all of the environmental variables more closely in LINEATION. This same pattern shows up in the regressions of the DFs on the environmental variables (Schueler, 1979).

Nonetheless, there is also the explanation that the samples of females are more evenly distributed along the environmental variables, and thus have more variation among sites than the males do: 8 of 13 CCAs between sets of environmental variables are stronger when weighted by the number of females from the sites than when weighted by the numbers of males. This is not an unlikely difference, but like the differences in body size it (p. 41) is in the same direction as the hypothesized selective effect.

In each of the analyses the first CC axis had skin loadings almost exclusively from SPOTTING+MSF1 and various of the grid and metric variables that are correlated with it (Table 5). There were generally environmental loadings in the same sense as SPOTTING from low elevation, humid climate, and warm temperatures, and often from higher elevations nearby, little wind and small extent of water area. There is no reason to suppose that all these relationships are causal: SPOTTING increases to the southeast which has a warm, humid climate, low elevation, and more topographic relief.

The second CC axis is again constant: it always has its highest loadings from VENTRAL GLANDS+MSF2, lesser loadings in the same sense from the grid tibia glands characters, and a lesser loading in the opposite sense from LINEATION+MSF3. There is a loading in the sense of GLANDS from SPOTTING+MSF1 in the analyses which included the locality variables, and in the opposite sense in those with climate alone. Dorsal spot area loaded with GLANDS in analyses of females, but against it in analyses of males. The loadings from the environmental variables emphasize the size of adjacent bodies of water, the absence of fields, cold and humid estimated climates or local NO FOG and FOG & SNOW.

The third CC axis is quite consistent across the different environmental variable sets, but is more variable in the skin variables. It generally has its highest skin loading from LINEATION+MSF3. In the local climate analyses, SPOTTING loaded in the opposite sense and, for females, LINEATION loaded weakly. Other variables that often contributed to the third CC were FEMUR LINE, in the sense of LINEATION, and DARK FEMUR BACKGROUND+MSF15, in the opposite sense. TIBIA LINE loaded with LINEATION in the female analyses and against it in the male analyses. The conspicuous feature of the environmental loadings for this axis is the invariably substantial loading from MARSH AREA, and the general loading of RIVER WIDTH and LAKE SIZE, in the same sense as LINEATION; WETLAND, SWAMP, FOREST, and WATER AREA generally load in the opposite sense. In the cases where SPOTTING loads with LINEATION (local climate analyses) WARMTH+PC1 loads against LINEATION, and when SPOTTING loads in the opposite sense WARMTH+PC1 loads with LINEATION, so that there is a tendency for SPOTTING and LINEATION to be associated in colder sites.

Table 14. Canonical correlations of skin and environmental variables.

Variable type	Specimens	Squared CC (variation explained)				
		1	2	3	4	5
DFs among localities		85%	69	47	43	27
CCs BETWEEN SKIN AND ENVIRONMENTAL VARIABLES						
Locality	pooled	69%	50	30	23	17
	male	68	50	35	22	21
	female	75	54	37	31	28
Estimated Climate	pooled	79	47	29	20	16
	male	80	52	27	26	17
	female	81	48	36	24	17
Estimated Climate & Locality	pooled*	81	64	39	32	19
	male	82	64	39	31	30
	female	82	67	45	41	33
Local Climate	pooled	71	37	21	12	8
	male	72	43	19	15	9
	female	72	39	29	17	14
Local Climate & Locality	pooled	80	57	34	30	20
	male	82	59	36	30	28
	female	81	61	43	38	33
CCs BETWEEN SETS OF ENVIRONMENTAL VARIABLES						
Locality With est. Climate	pooled	96%	71	56	46	37
	male	96	75	50	48	30
	female	96	73	61	50	38
Locality With local Climate	pooled	82	62	57	36	20
	male	85	66	50	42	14
	female	81	68	60	38	26
Est. clim. With local Climate	pooled	93	84	74	21	16
	male	94	84	71	21	14
	female	94	83	76	19	18

*this is the analysis for which CC scores were computed.

Table 15. Skin coefficients for canonical correlation scores.

Variables	Mean	s.d.	Standardized Coefficients		
			CC1	CC2	CC3
		MULTISTATE FACTORS			
1 SPOTTING	0.0	1.0	0.57160	-.04335	-.53792
2 VENTRAL GLANDS	0.0	1.0	-.08337	-.66323	-.05524
3 LINEATION	0.0	1.0	-.11720	0.27039	-.70054
4 HEAD SPOTS	0.0	1.0	-.01439	0.02425	0.05528
5 TIBIA BROKEN BARS	0.0	1.0	-.08810	0.03412	-.01158
6 FEMUR DIAGONAL BARS	0.0	1.0	-.00529	-.06194	0.02702
7 TIBIA HALF BARS	0.0	1.0	-.06381	0.02977	-.11649
8 FEMUR HALF BARS	0.0	1.0	-.16745	-.02185	-.19993
9 TYMPANUM SPOT	0.0	1.0	-.03783	-.02766	-.01676
10 SPOT OUTLINING	0.0	1.0	0.01511	-.04587	0.13613
11 NOSTRIL SPOT	0.0	1.0	-.01094	-.03035	-.05917
12 NOSE SPOT	0.0	1.0	-.07085	0.03209	-.13431
13 DUSKINESS	0.0	1.0	0.01275	0.01801	-.01183
14 FEMUR BROKEN BARS	0.0	1.0	-.02533	-.06141	0.02437
15 FEMUR BACKGROUND	0.0	1.0	-.02325	-.09597	0.04647
16 MERGED DORSAL SPOTS	0.0	1.0	0.12118	-.08680	-.03399
17 SPOT BILINEARITY	0.0	1.0	0.03945	-.00237	-.09774
18 LATERAL LINEATION	0.0	1.0	0.12209	0.08162	-.05972
		METRIC VARIABLES			
NOSE SPOT LENGTH	0.5166	0.3196	0.03636	0.02871	0.08202
LONGEST GLAND LINE	1.3729	0.1556	0.08369	-.05746	-.02571
LONGEST SPOT	1.0884	0.1451	0.01317	-.04938	0.07655
MIDFEMUR SPOTTING	1.2167	0.1275	0.10372	0.07013	-.00594
FEMUR LINE	0.8560	0.4820	0.16152	-.08563	-.14295
TIBIA LINE	1.3010	0.3314	0.09406	0.08153	-.06948
		GRID VARIABLES			
DORSAL SPOTTED	0.6849	0.0750	-.19357	0.11617	-.04820
DORSAL UNSPOTTED	0.8201	0.0628	-.14995	0.08929	0.03617
UNSPOTTED-NO GLANDS	0.7695	0.0865	-.02234	0.22264	-.32127
UNSPOTTED-GLANDS	0.7072	0.1199	-.02491	-.10550	-.45677
SPOTTED-NO GLANDS	0.7200	0.0991	0.10998	0.10848	0.37093
SPOTTED-GLANDS	0.6700	0.1204	0.09346	0.03028	0.16361

Table 16. Correlations between DFs and CCs.

			Environmental Canonical Correlation Axes			SKIN
		DF	1	2	3	SIZE
DISCRIMINANT FUNCTIONS	Spotting	1	-.99	-.06	0.01	0.35
	Glands	2	0.03	-.99	-.09	-.12
	Size & Lineation	3	0.06	0.12	-.65	0.62
		4	-.05	0.06	-.71	-.51
	Lineation	3&4	0.05	0.11	-.98	0.00
		5	0.00	-.07	0.02	-.16
	Skin size		-.30	0.17	-.02	1.00

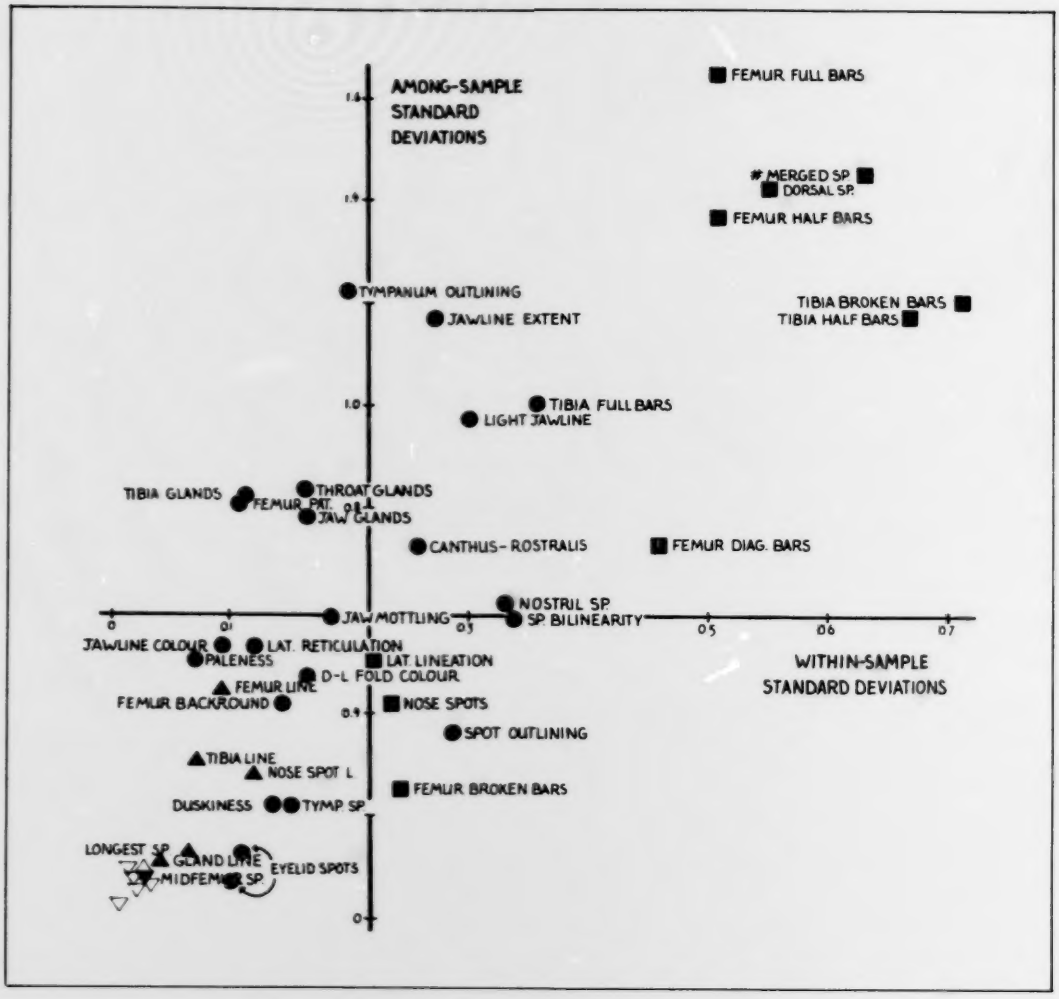

Figure 21. Kluge-Kerfoot diagram for skins.

This is a plot of within-sample vs. among-sample standard deviations for the metric (▲ and grid (▽) variables and the multistate characters with defined character states (●), and which are counts (■). The axes are at the means.

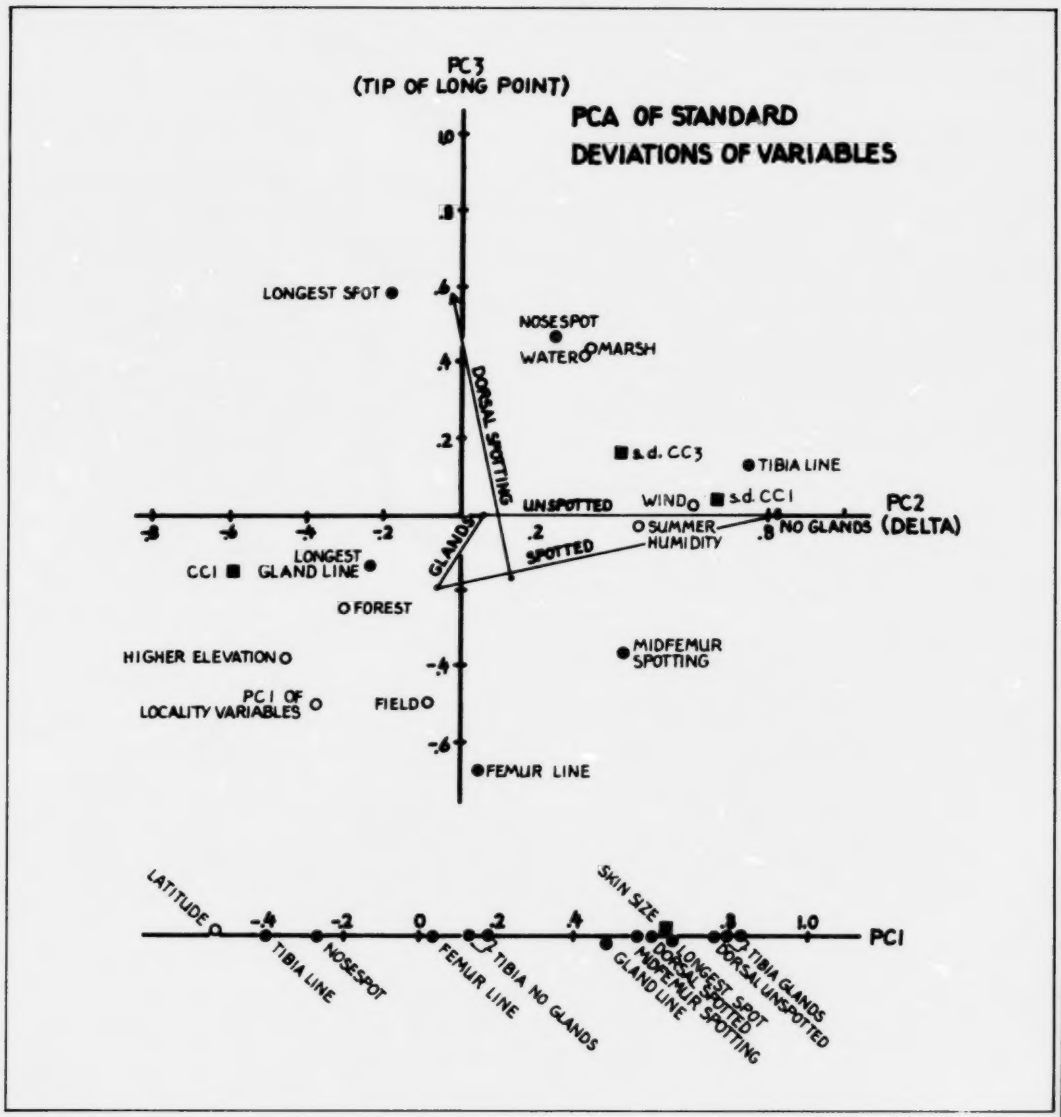

Figure 22. PCA of variation in variability.

This shows the loadings of characters (dark circles) on PC1 (below) and PCs 2&3 (above), and correlations of some skin (squares) and geographic and environmental (open circles) variables with the PCs. The arrow points from DORSAL UNSPOTTED to DORSAL SPOTTED, and the tibia grid variables are at the apicies of the broken lines, which connect variables that express one state of spotting or glands on the grid.

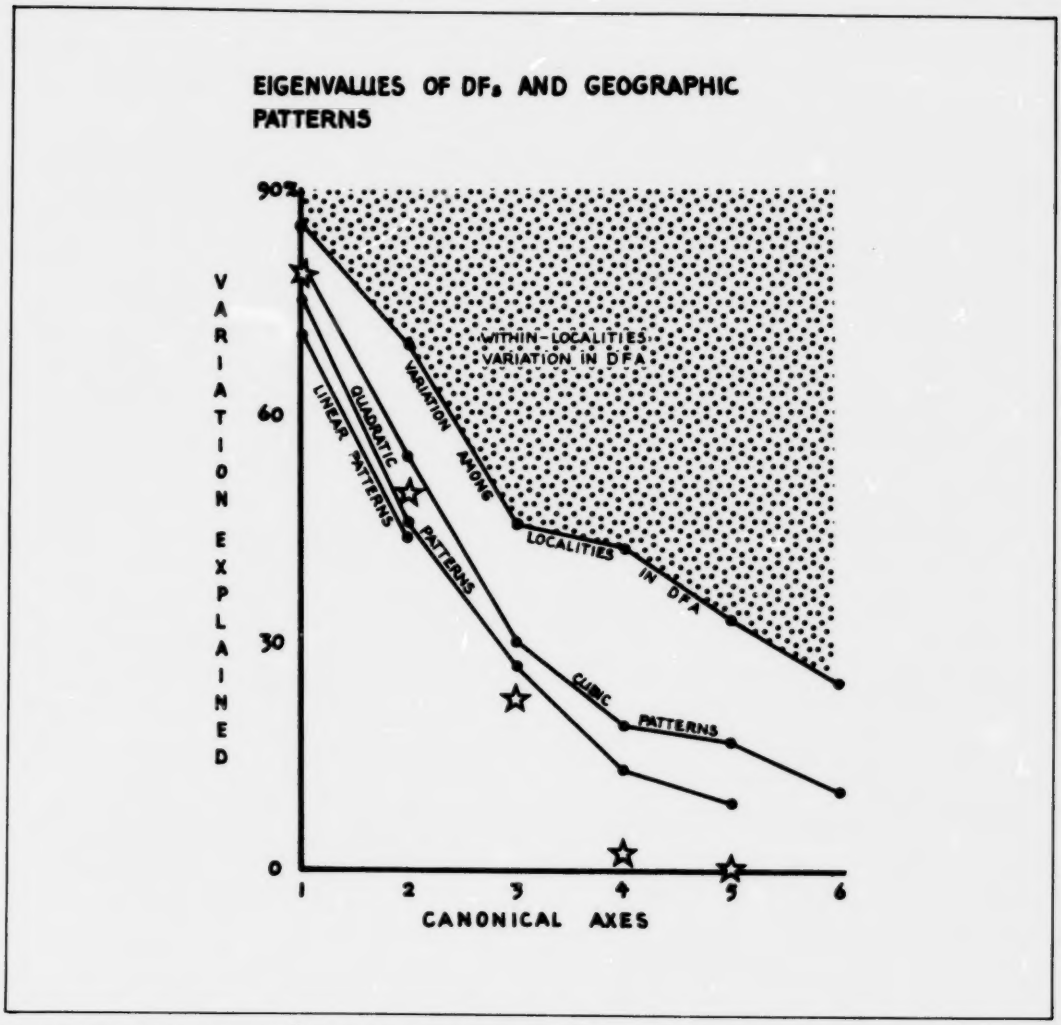

Figure 23. Relationships between DFA among localities and CCAs of geographic patterns.

The among-localities DFA and the skin-geographic CCAs both produce series of uncorrelated composite skin variables with minimal deviations from predicted values: in the first case from group means and in the other from trend surfaces. This figure compares the variation accounted for in these analyses (the square of the canonical correlations), since the TSs cannot account for variation within the samples, and the DFs account for the among-sample variation as efficiently as is possible. The variation can be said to be geographic to the extent that the TS accounts for most of the among-localities variation. The comparison is not exact, because the CCAs are based on all of the specimens, and the DFA is based on those in samples of 10 or more. The stars show the variation in each DF explained by correlation with the corresponding cubic geographic pattern. The fact that each of the first three DFs is highly correlated with the corresponding geographic pattern establishes the identity of these axes of variation in the two analyses.

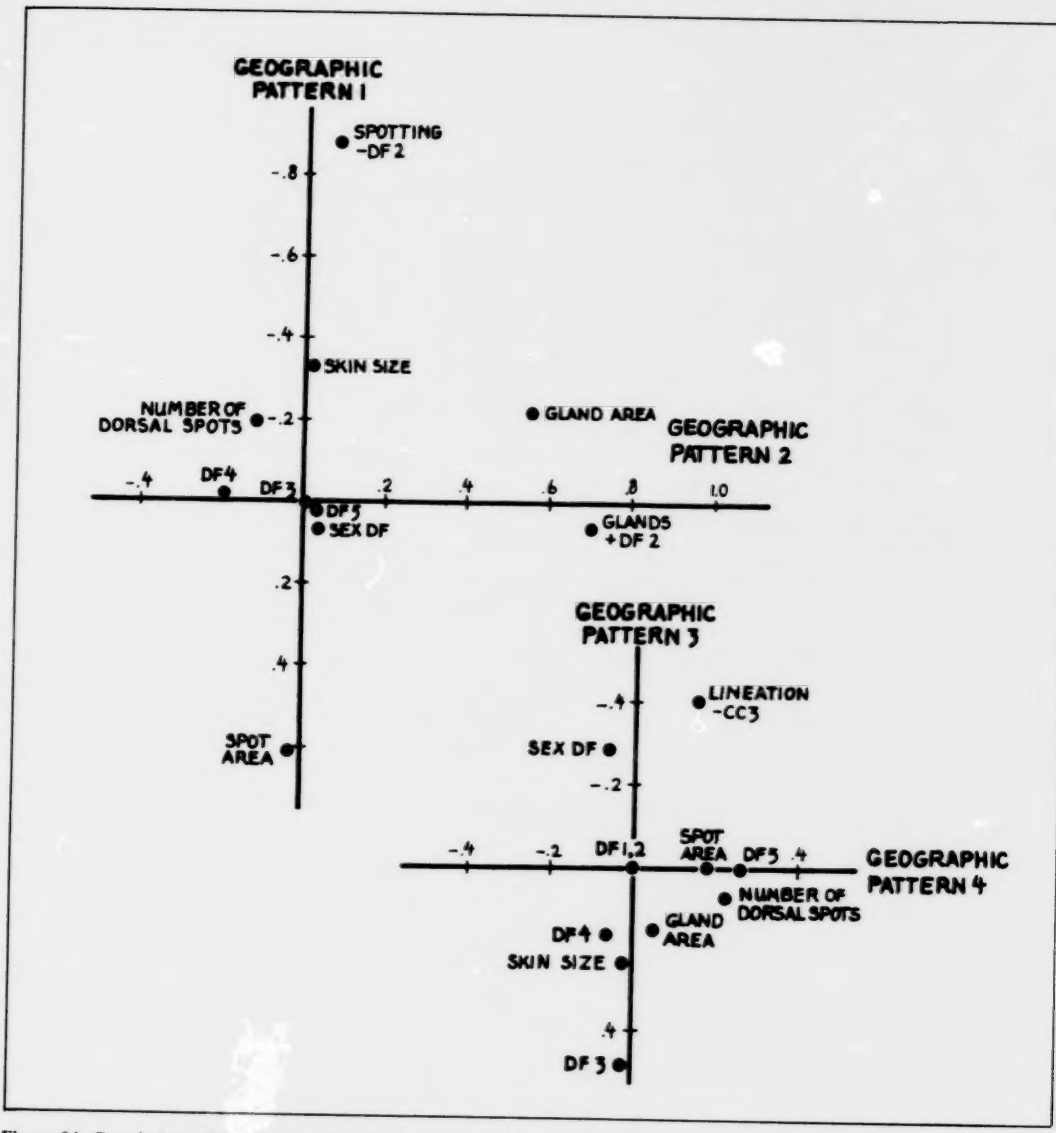

Figure 24. Correlations of some composite variables with cubic geographic patterns.

These are correlations of specimen values with the values of the TSs at the point of collection, so the strength of the correlation depends on both the extent to which the geographic pattern represents geographic variation in the variable and how much among-localities variation there is (compare Figure 23). LINEATION-CC3 is defined in p. 43 ff.

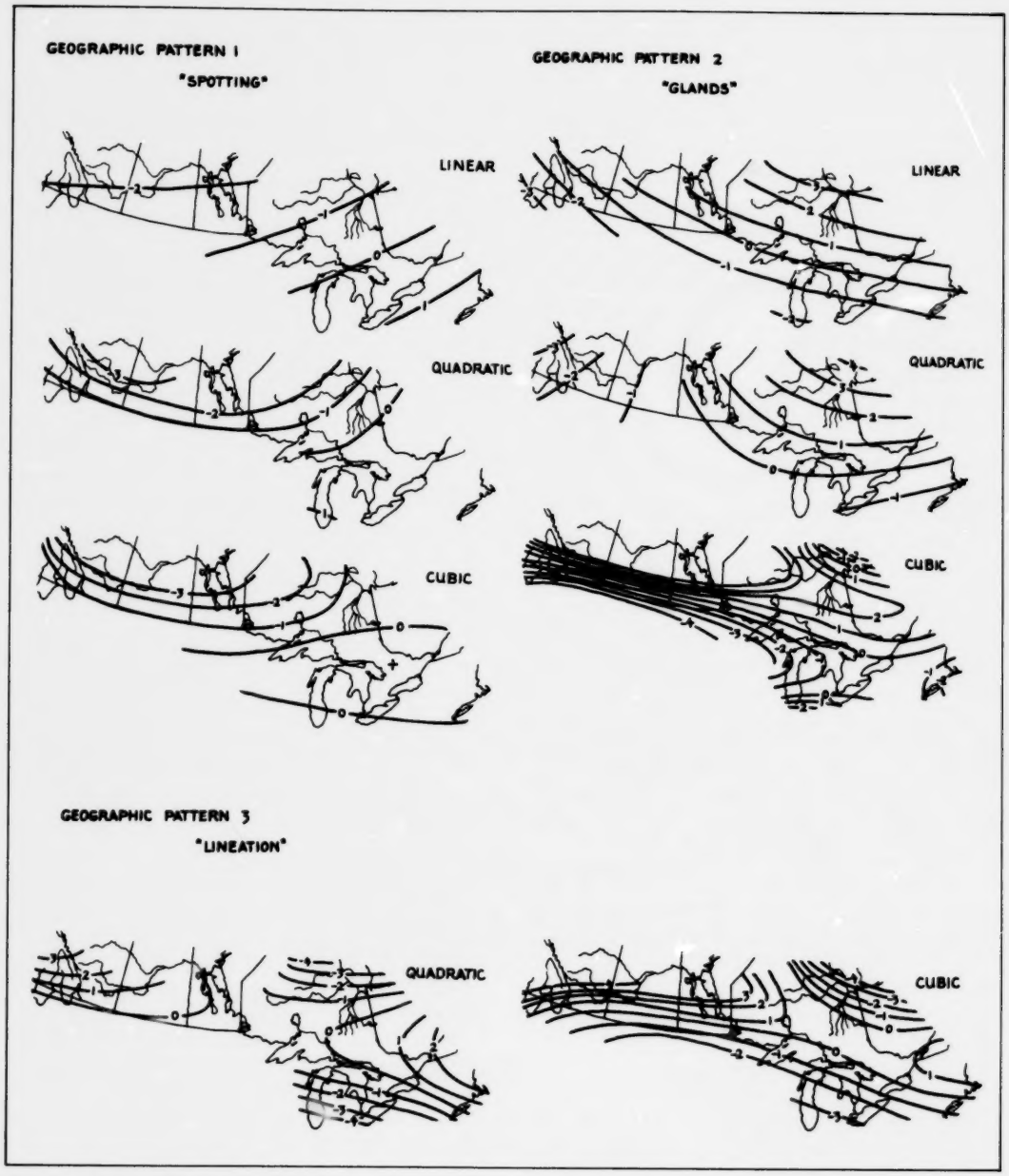

Figure 25. Patterns of geographic variation.

These are maps of the first 3 patterns of geographic variation for the linear, quadratic, and cubic analyses. The contour intervals are standard deviations of the standardized pooled data. The patterns are distorted by the use of different projections in the analysis and the base map: this is why the isolines in the linear maps not straight. The coefficients of these maps are listed in Appendix 2.

Part IV: Replication

This Part checks results previously obtained by repeating analyses on separate sets of data. When I began the present study I did not plan to repeat it on other specimens, and most of the preceding analysis had been completed before I realized that I had data available for a major replication in measurements of 864 NMC and CU fluid-preserved specimens I examined in 1972-3, just before I began to score the skin specimens. This includes only a few of the characters scored on the skins, and the dermal glands and LINEATION characters are scarcely represented, but there are enough characters to test the spatial stability of some of the patterns of variation found in the skin specimens.

Spotting and Glands Replication: Fluid-Preserved Specimens

The characters taken on both the skin and fluid-preserved specimens are three metric characters (SKIN LENGTH, LONGEST DORSAL SPOT, and LONGEST GLAND LINE), four spot counts (NOSE SPOTS, LEFT & RIGHT EYELID SPOTS, and NUMBER OF DORSAL SPOTS) and four other multistate characters (TYMPANUM OUTLINING, LATERAL SPOT RETICULATION, FEMUR BACKGROUND COLOUR, and FEMUR PATTERNING). I compared geographic variation in SPOTTING and GLANDS in the two sets of specimens by using trend surfaces based on the geographically more extensive set (fluid-preserved) to predict variation in the skins, and comparing the variation explained by this TS with the variation explained by a TS of the same order based on the skins.

1) I made estimates of SPOTTING and GLANDS which could be computed for the fluid-preserved specimens by stepwise regression of CC1 and CC2 scores of the skins on the replication variables (and, to allow for nonlinear relationships, their squares and square roots); these variables are called REPLICATION SPOTTING and REPLICATION GLANDS.

2) I described geographic variation in the fluid-preserved specimens by TSA of REPLICATION SPOTTING & GLANDS on the fluid-preserved specimens.

3) I compared this geographic variation to that in the skin specimens as the percentage of the variation in CCs 1 & 2 explained by a TSA of the skin specimens accounted for by the TS based on the fluid-preserved specimens. Thus if a cubic TSA of the skin specimens accounted for 60% of the variation in a variable and these data were correlated with the TS from the fluid-preserved data at 0.55 (30% of variation explained), then the success of the replication would be 50%. The comparison could be made only from the fluid-preserved to the skin specimens because the fluid-preserved specimens came from a wider geographic area than the skins (Figure 26).

In the regression estimate of CC1 the first variable to enter was the square root of FEMUR PATTERN (67% variation explained) followed by the square root of TYMPANUM OUTLINING (76%), the square root of LATERAL LINEATION (78%), the NUMBER OF DORSAL SPOTS (80%), and the sum of NOSE SPOTS and EYELID SPOTS (80%). The two spot counts loaded in an opposite sense to the other variables.

Replication of CC2 was less successful, as the only variable which bears directly on GLANDS is LONGEST GLAND LINE: the square roots of this and of SKIN LENGTH combine to explain 26% of the variation in CC2, and inclusion of the square roots of TYMPANUM OUTLINING (against GLANDS) and of LATERAL LINEATION (with GLANDS) increased the variation explained only to 29%.

REPLICATION SPOTTING and REPLICATION GLANDS are uncorrelated ($r = 0.02$ in the fluid-preserved sample, and 0.04 in the pooled sample). REPLICATION SPOTTING has the marked negative relationship with NUMBER OF DORSAL SPOTS seen in the skin sample ($r = -.27$; this is at least influenced by the contribution of DORSAL SPOTS to REPLICATION SPOTTING).

The geographic patterns resulting from TSA of these variables, and of NUMBER OF DORSAL SPOTS, were similar to those in the skin specimens (Table 17), and confirm that at least the quadratic patterns are robust enough to appear in samples collected at largely different sites, decades apart, preserved differently, and scored for different sets of characters. There is little similarity in the percentage of geographic variation in the two data sets: less of the SPOTTING, far less of the GLANDS, but more

of the variation in DORSAL SPOTS is explained by TSA in the fluid-preserved than in the skin sample. There is no similarity in the patterns of size variation: this is doubtless because the western skins were collected in the spring and comprised only adults, but the fluid-preserved collections were made later in the season and contained both adults and newly transformed juveniles.

Lineation Replication

The replication variables for the fluid-preserved specimens are quite unrelated to LINEATION variation (the best multiple regression absorbed only 19% of the variation in CC3), but since this is the canonical axis most closely related to the DF between the sexes some check on the robustness of the patterns of variation seen in the pooled skin samples can be made by comparing the geographic and environmental relations of CC3 for males and females. The habitat relations of LINEATION might, for instance, reflect habitat selection by the sexes rather than among-localities differences in the frogs.

I computed linear, quadratic, and cubic TSs and stepwise multiple regressions of the first 5 environmental variables entered in the path diagram analysis (Figure 32) for the CC3 scores of males and females, and used variation in each sex to predict variation in the other.

The results in Table 18 show that the replication is much more successful for the environmental variables (>90%) than for the geographic variables (ca. 60%). The patterns of variation with the environmental variables therefore seem to be the same in each sex even though the mean values of CC3 for the sexes are quite different (±0.12 s.d. of the pooled data), and LINEATION is seen to be more closely related to habitat variation than to geography.

Eyelid Spot Asymmetry

Among 782 leopard frogs from Ontario, Illinois, Utah, Florida, and Mexico, Bresler (1964) found 20 which had a spot only on one eyelid, 16 of which lacked the spot on the left eyelid, a moderately unlikely distribution. In Table 19 Bresler's data are compared with those from the present study, and it is evident that the relationship in the available *Rana pipiens* is in the opposite direction to that found by Bresler, though not significantly so. When the *R. pipiens* data are pooled with Bresler's other specimens there are 23 specimens with only the right eyelid spot, and 19 with only the left, so there is no overall asymmetry. The low probabilities for the contingency tables in Table 19 are due to the absence of both eyelid spots more often than expected by chance: the influence of HEAD SPOTS+MSF4.

'Estimated' and 'Local' Climate Variables

Four of the estimated and local climate variables are weakly correlated (Table 12), so it is not surprising that they often have quite different relationships with the skin variables, although 3 of the first 5 DFs do have their strongest climatic correlations with the same climate PC in both variable sets (DF1 with ARIDITY, DF2 with NO FOG, and DF4 with FOG & SNOW). To see if the differences between the variable sets were due to errors introduced by the great distances between the collection sites of some of the specimens and their 'local' data I divided the specimens into two groups on the basis of the mean distance from which the local data associated with them came, and compared the explanatory power of the local and estimated variables in stepwise inclusion into multiple regressions of the first 5 DFs. If the differences between the local and estimated data are errors introduced by the TS estimates and local data from long distances, then the 'near' local data should give the best explanation of the skin data. If, on the other hand, the climate variables do not measure environmental factors to which leopard frogs respond, the estimated data, which represent a simplified geographic pattern, might be expected to give the best fit to the skin data.

There were 415 specimens in the 'near' group (mean distance < 38 km) and 413 in the 'far' group (mean distance > 38 km). The results for three steps show that the 'near' local data do not give a better explanation for the skin data; this is the case only for DF5. The 'near' estimate provides the best fit 3 times, and the 'far' local data once, so it is at least safe to say that the differences between the two variable sets is not that the estimates are inaccurate or that some of the local data are contaminated by inaccuracies arising from the distances between the stations and the collection sites. Perhaps the geographic smoothing of the estimated data by the TS and of the 'far' local data by the averaging of distant

Table 17. Replication of geographic variation in SPOTTING and GLANDS.

Analysis	Variation explained by TSA of: original variable (skins)	replication variable (fluid-pres.)	fluid-pres. specimens among skins	Success of replication (col. 3/col. 1)
		SPOTTING+CC1		
Linear TSA	69%	30	68	99%
Quadratic TSA	75	42	59	78
Cubic TSA	79	48	59	75
		GLANDS-CC2		
Linear TSA	34	3.0	31	92
Quadratic TSA	37	4.8	33	89
Cubic TSA	50	5.7	30	60
		NUMBER OF DORSAL SPOTS		
Linear TSA	6.5	11	6.3	96
Quadratic TSA	7.8	21	6.3	81
Cubic TSA	13	26	5.5	43
		SIZE		
Linear TSA	9.9	0	Nil	Nil
Quadratic TSA	17	5	Nil	Nil
Cubic TSA	18	5	0.0	0%

stations tends to remove microclimatic peculiarities of the climatic station sites and gives a better expression of the regional climatic variation to which the frogs are exposed.

This failure of 'near' local variables to provide the best explanation of the skin data suggests that no causal climatic significance can be assigned to correlations between the skin and climate variables; the skins vary with climate, but there is no evidence here that they vary because of climate.

Table 18. LINEATION replication between sexes.

Analysis		Variation explained by regression prediction based on specimens of the other sex, and success of replication			
		MALE		FEMALE	
GEOGRAPHIC TREND SURFACE ANALYSIS					
	Linear	2.6%	60%	5.2%	59%
Trend	Quadratic	7.9	69	8.8	63
Surface	Cubic	15	68	10	37
STEPWISE REGRESSION ON ENVIRONMENTAL VARIABLES*					
Step 3 SWAMP AREA		12	100	16	100
Step 4 RIVER WIDTH		13	99	17	99
Step 5 WARMTH		15	93	16	91

*MARSH AREA and FIELD AREA entered in Steps 1 & 2 in both cases.

Table 19. Leopard frogs with asymmetrical eyelid spots. Tests of frequencies from Sokal and Rohlf (1969).

ALL OF THE LEOPARD FROGS EXAMINED BY BRESLER (1964).

		RIGHT EYELID SPOT	
		Present	**Absent**
LEFT EYELID SPOT	Present	733	4
	Absent	16	29

G=16.5 (for entire table) $p<0.005$; 2-Tailed binominal test for left-right symmetry: $p=0.012$

SKIN SAMPLE.

		RIGHT EYELID SPOT	
		Present	**Absent**
LEFT EYELID SPOT	Present	815	6
	Absent	2	5

G=37.6 (for entire table) $p<0.005$; 2-Tailed binominal test for left-right symmetry: $p=0.29$

FLUID-PRESERVED SAMPLE.

		RIGHT EYELID SPOT	
		Present	**Absent**
LEFT EYELID SPOT	Present	850	7
	Absent	5	2

G=10.4 (for entire table) $p<0.005$; 2-Tailed binominal test for left-right symmetry: $p=0.39$

ALL *RANA PIPIENS* POOLED (skin, fluid-preserved, Bresler's (ROM) Ontario sample (88), 298 MCZ & 64 NMC specimens).

		RIGHT EYELID SPOT	
		Present	**Absent**
LEFT EYELID SPOT	Present	2103	15
	Absent	8	16

G=115 (for entire table) $p<0.005$; 2-Tailed binominal test for left-right symmetry: $p=0.21$

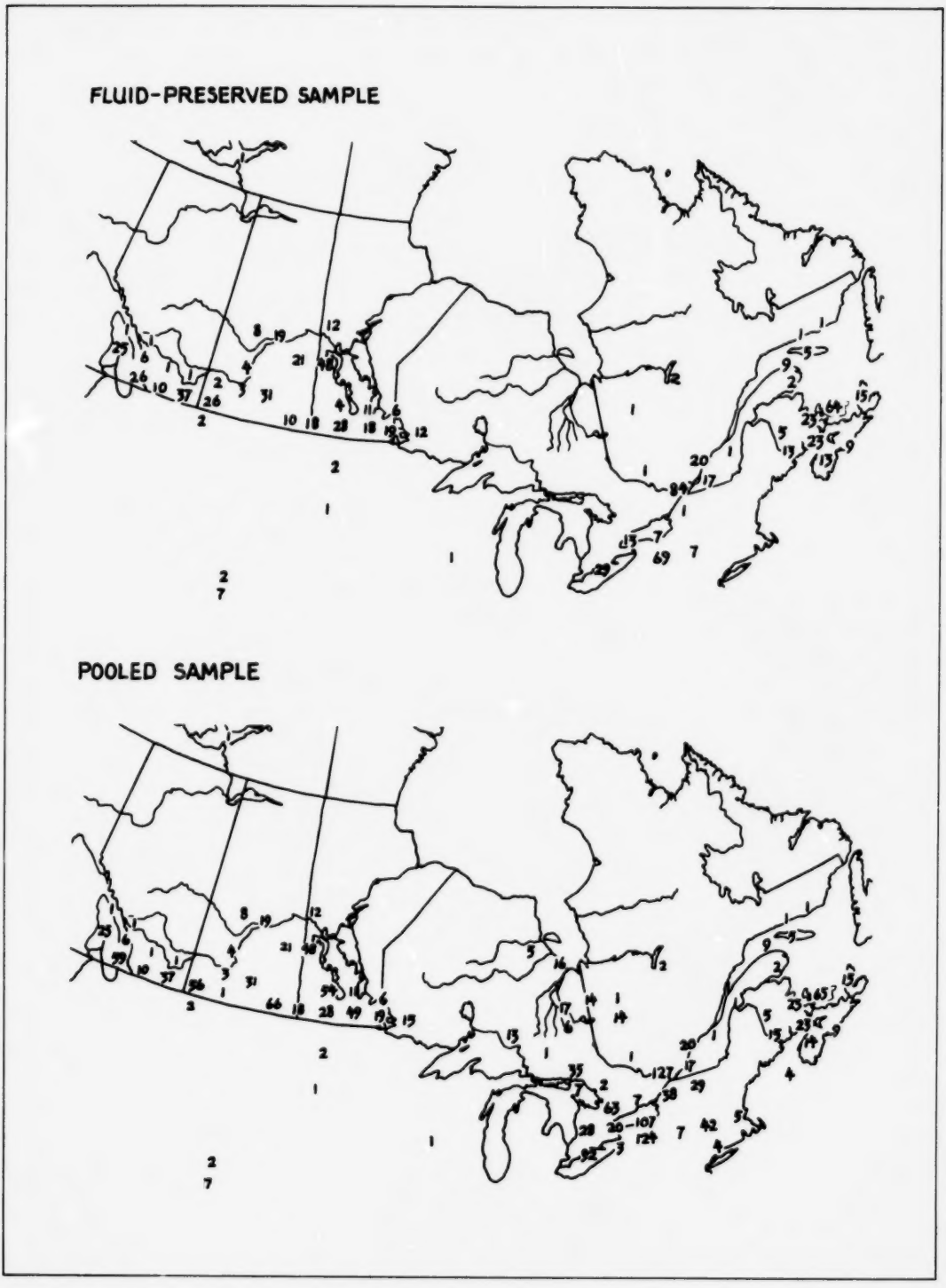

Figure 26. Geographic origin of specimens used in replication analyses.

This shows the number of specimens in the fluid-preserved (above) and pooled (skin + fluid-preserved; below) samples, summed over 1°N by 2°W quadrats of latitude and longitude.

Part V: Adaptation — General Summary & Discussion

This Part considers adaptive explanations for the variation described by (1) the first three CC variables (SPOTTING, GLANDS, and LINEATION) (2) the associated estimates of parameters (SPOT AREA, GLAND AREA, the NUMBER OF DORSAL SPOTS), and (3) the size of the specimens.

The fact that much of the variation among localities can be explained in broad geographic terms may be evidence that it is selectively ordered by environmental factors (Gould & Johnston, 1972; Clarke, 1975), but in the absence of clear-cut causal hypotheses it is hard to decide when a specimen-environment correlation is the result of covariation with a genuinely causal factor or is merely spurious.

It is also possible that geographic variation is not due to evolutionary responses of populations to the environments they presently inhabit, but to responses to a much more restricted and temporally prolonged subset of these environmental conditions in which their ancestors survived one or several Pleistocene glaciations. Such historical effects would be manifest by a pattern of variation that is dependent on geographic rather than environmental variations: particularly longitude rather than latitude (since glacial refugia would have been aligned east-west south of the ice, and attributes that show persistant historical effects should not have changed much as colonizing populations spread north; Bleakney, 1958b). Such attributes might also be expected to show hybridization (Schueler & Rising, 1976) where descendents of the different refugia come into contact (Cook, in press). Endler (1977), on the basis of laboratory experiments and a mathematical model of gene flow, doubted that post-Wisconsin contact zones would still be evident, but Simpson and Haffer (1978) claimed to find them all over Amazonia.

Spotting

SPOTTING, as CC1, is highly correlated with SPOT AREA (r=0.91 among locality means, 0.64 within samples). The environmental relations of these variables are also very similar: the correlation between the path coefficients for Figure 28 and the comparable SPOT AREA diagram is 0.88. The highest geographic correlation for both is with Pattern 1 (Figure 24). In the path analyses, LATITUDE both enters first and has the highest path coefficient, while WARMTH and WATER AREA enter next, and these three, explaining about 85% of the among-localities variance, have the only substantial path coefficients. The relationship of SPOTTING with LATITUDE and WARMTH is indistinguishable from the increased SPOTTING in the southeast seen in Geographic Pattern 1 (Figure 25), and the western fluid-preserved sample demonstrates a southward decrease in spotting in the Great Plains (Figure 27). Within drainage basins (Great Lakes, James Bay, Hudson Bay, and Atlantic) there is no relationship between SPOTTING and the climate variables, so most of the variation in SPOTTING is described by general geographic tendencies due, presumably, either to derivation from different glacial refugia or to broad climatic or habitat differences. Although among the skins the geographic component is associated with latitude rather than longitude and there is a strong relationship between SPOTTING and WATER AREA the correlations of REPLICATION SPOTTING with herpetofaunal regions (Cook, in press, and in prep.) are just as strong as its correlations with geography (Table 20), so the possibility of historically determined variation cannot be excluded.

The environmental correlates of SPOTTING resemble 'Gloger's Rule' variation (Allee & Schmidt, 1951): frogs from warm and moist areas have more extensive dark pigmentation than those from cold and dry areas. The negative correlations of SPOTTING with WATER and MARSH are maintained within drainages, so open marsh habitat and large bodies of water result in reduced SPOTTING, probably because they provide paler, less patterned backgrounds in the summer, and perhaps also in the winter. Gloger's Rule has not been adequately explained, but it doubtless results from greater crypsis of dark animals in the deeper shadows and more moist and humid backgrounds resulting from the ranker vegetation of less arid areas, and may combine elements of dark energy coloration in shaded environments where refuge from the sun is always available (Papageorgis, 1975).

Such a pattern is not at all a general rule in *Rana:* the frequency of snout spotting increases to the north in *R. utricularia* (Pace, 1974), as does

dark pigmentation in *R. sylvatica* (Martof and Humphries, 1959; Schueler, 1973), *R. palustris* (Schaaf and Smith, 1970; pers. obs.), *R. clamitans* (Mecham, 1954; pers. obs.), and perhaps *R. aurora* and *R. pretiosa* (pers. obs.) so this pattern probably results more from the habitats used by *R. pipiens* than from general adherence to Gloger's Rule. *R. palustris*, in particular, has reduced dorsal spotting in the southwest of its range, and north along the Atlantic coastal plain to Cape Cod, and the Mississippi River west to Illinois (Schaaf & Smith, 1970; Schueler, 1979, Figure 1-4), and perhaps all that these habitats have in common is that *R. palustris* is more aquatic there than elsewhere. The northward reduction in spotting in *R. pipiens* may also be due to the use of a more aquatic niche north and west of the ranges of *R. catesbeiana*, *R. clamitans*, and *R. septentrionalis* (Schueler, 1973, 1975). Even without such 'ecological release' terrestrial frogs of drier areas may spend more of their time near and in water because of the lesser area of habitat with saturated soil available to them (Tracy, 1975, 1976), so it may be that much of the variation in SPOTTING parallels the tendency for less-patterned coloration in aquatic frogs found in the among-species analysis.

Number of Dorsal Spots (NDSP)

The number of dorsal spots has not been included in the analyses described above, in order to compare variation in NDSP, by itself, with the composite spotting variables. NDSP is negatively correlated with SPOT AREA and SPOTTING+CC1 among the weighted locality means (-.20, -.42), and within samples is weakly negatively correlated with SPOTTING+CC1 (-.16) but positively correlated with SPOT AREA (0.13) and GLANDS-CC2 (0.11) but not with GLAND AREA (0.02). About a half of the among-locality variation in NDSP in the skin specimens is described by a cubic TS opposite to that of SPOTTING, and 17% of the variation in the pooled sample is accounted for by a very similar cubic pattern (Table 17, Figure 29). The fluid-preserved sample includes many specimens from the west, and the steep increase in NDSP in the northwest of Figure 29 (and the related high correlation with sympatry with *Bufo boreas*; Table 20) is the only evidence of stepped clinal variation encountered in the present data, and may be a sign of the derivation of far-western *Rana pipiens* from populations in which dorsal spots are smaller and more rounded than in the eastern stocks.

If the function of these spots is disruptive, to so stimulate the eyes of predators with small, dark, roundish shapes that they do not see the outline of the frog, then it would be expected that there would be greater variation within populations than among them, since any approach to a fixed disruptive pattern would allow predators to form a searching image for the prevalent arrangement of spots (Clarke, 1969). This expectation is met in the negative among-sample correlation between NDSP and SPOT AREA, which suggests that selection for disruptive effect tends to maintain a relatively fixed length of spot edge to compensate for variation in spot area. The negative within-sample correlation between SPOTTING and NDSP and the positive within-samples association between SPOTTING and MERGED SPOT PRIMORDIA (r=0.20) suggest that at least part of this compensation may be due to the merging of spot primordia in populations with large spot area. The idea that apostatic selection for diverse disruptive patterns determines NDSP is also supported by the 'character displacement' between spot number and the frequency of burnsi in Minnesota (Merrell, 1965; p. 33), and this cannot be due merely to the merging of primordia in specimens with extensive spots, because Merrell's 'spots' are the same as my 'primordia'.

Little sense can be made of the path diagram for NDSP (Figure 30). WATER AREA decreases both NDSP and SPOTTING; WETLAND SIZE reduces NDSP and MARSH and SWAMP AREA increase it, but all have little or negative effect on SPOTTING. These relationships may prove useful for comparison with studies of microgeographic variation in spotting in *Rana pipiens*, but they are not interpretable now.

Glands

Like SPOTTING and SPOT AREA, GLANDS and GLAND AREA are highly correlated (r=-.81 among locality means, -.85 within groups) and share a common pattern of variation on the environmental and geographic variables (Figures 31&32; r=0.79 between path coefficients for Figure 32 and the comparable GLAND AREA diagram). The multiple regression shown in the path diagram explains a little less of the variation than a cubic TSA, and the path coefficients have

Table 20. Correlations between replication variables and herpetofaunal regions.

Herpetofaunal regions are defined by sympatry with widespread species of *Bufo* (Cook, in press), and the correlations are with variables assigned a value of '1' for sympatry and of '0' for allopatry.

			SYMPATRY WITH		
			Bufo americanus		
	Latitude	Longitude	*americanus*	*hemiophrys*	*Bufo boreas*
REPLICATION SPOTTING	0.57	0.66	0.67	-.59	-.22
# OF DORSAL SPOTS	-.31	0.26	-.29	0.15	0.30
REPLICATION GLANDS	-.08	0.13	-.01	0.14	-.06

Variation in spotting variables explained by stepwise regression on cubic geographic and *Bufo* sympatry variables:

REPLICATION SPOTTING

STEP	GEOGRAPHIC	*BUFO* SYMPATRY
1	44% (E)	45% (*americanus*)
2	47% (E**3)	(no entry)

NUMBER OF DORSAL SPOTS

STEP	GEOGRAPHIC	*BUFO* SYMPATRY
1	10% (N)	8.9% (*boreas*)
2	15% (N**2)	13% (*hemiophrys*)

a simple environmental interpretation: the variables which influence the extent of dermal glands are those which determine the exposure of the frogs to submergence in water. GLANDS increases with RIVER WIDTH, WATER AREA, LAKE SIZE, and LATITUDE, and it decreases with FIELD AREA and ARIDITY. The large coefficients for LATITUDE and the inconsequential ones for WARMTH suggest a more direct effect of latitude than low temperatures; perhaps the longer winters in the north effect the increase in GLANDS by prolonging the frogs' exposure to water during hibernation. These relationships clearly support an hypothesis of an electrolyte balance or infection prevention function for increased (serous?) glands rather than a thermoregulatory function due to selection for increased mucous flow in thermally stressed populations. The pigmentation variation associated with GLANDS also supports this hypothesis: glandular populations tend to be relatively aquatic in colour pattern (Table 15; Table II-6 in Schueler, 1979).

Table 21. Variation in GLAND AREA and SPOT AREA.

	SPOT AREA	GLAND AREA
Mean	38.4%	42.5
Pooled within-groups s.d.	6.0	11.5
Range of within-groups s.d.'s	3.6-9.4	7.4-11.6
Total s.d.	8.3	16.3
Probability that variances of groups are equal (Bartlett-Box test)	0.0002	0.105
Among-groups variation	49%	52%

Despite this fairly straightforward environmental interpretation of variation among the means of populations, there is great variability in GLANDS within populations. Half of the variation is within samples, the standard deviations of GLAND AREA within samples are uniformly about 11%, and there are two samples (Pillar Point and Webbwood) in which the range encompasses the means of all the samples (22-72%). SPOT AREA, on the other hand, has lower and more variable within-sample standard deviations (Table 21). This is much more variation than occurs within or among the populations of *R. palustris* or *R. utricularia* that I have examined (*e.g.*, the WSI axis in Figure 7). It suggests intense diversifying selection in environments which are effectively coarse-grained while

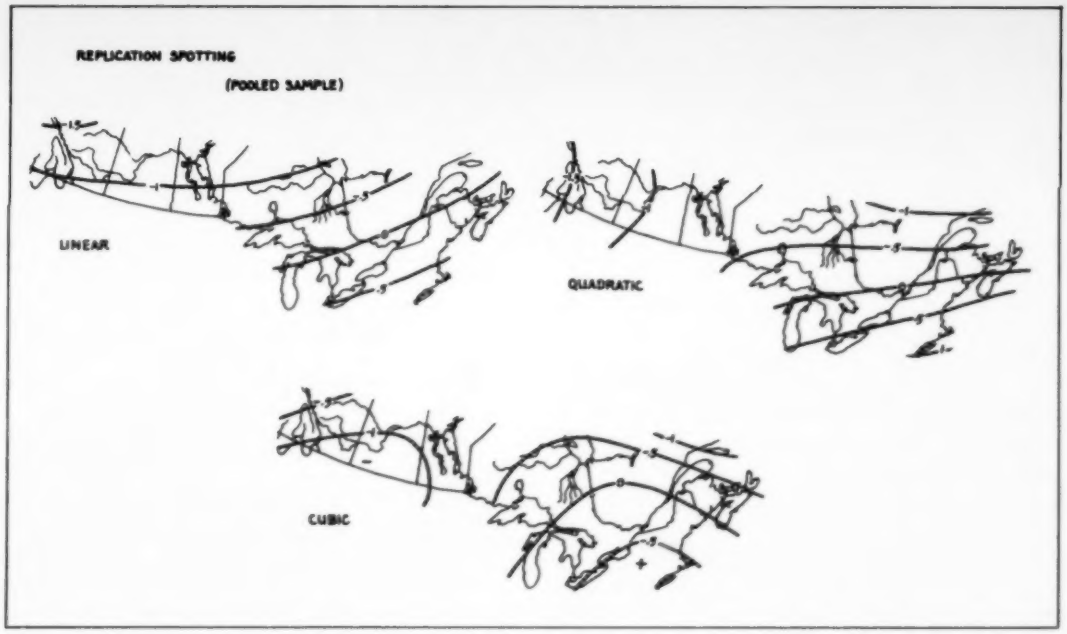

Figure 27. Geographic variation in SPOTTING: TSA of pooled samples.
This analysis is based on the REPLICATION SPOTTING variable among the pooled skin and fluid-preserved specimens. The scale is standard deviations from the mean of the skin sample (*i.e.* REPLICATION+SPOTTING is based on SPOTTING+CC1, which is standardized for the skins). The coefficients are:

	LINEAR	QUADRATIC	CUBIC
Constant	0.2496481	-1.781144	0.1919585
E	0.01366312	0.1114351	-.1316955
N	-.1347896	-.03141879	-.3358696
E**2		-.0009985952	0.006915447
E*N		-.004115907	0.007354369
N**2		0.003543508	0.02964047
E**3			-.00008240851
(E**2)*N			-.00002766772
(N**2)*E			-.00062902
N**3			-.0003889459

E=120-Longitude, N=Latitude-40

the selection is occurring, so that frogs remain within selectively homogeneous habitat patches during the selective mortality, but were mixed in my collections either because I collected in several patches or because the populations had reassembled after the selection. This scheme is not incompatable with the idea that much of the selection for GLANDS occurs during hibernation, and it suggests that selection is intense enough to be routinely observed in local populations (Berry & Crothers, 1968).

R. areolata (p. 5), *R. sylvatica* (Martof & Humphries, 1959), and *R. pretiosa* (pers. obs.) also show a northward increase in glands, though *R. palustris* may not (p. 5), and *R. aurora* has wider dorsolateral folds in the south where it is more aquatic (p. 4), so it may be that all these semi-terrestrial frogs are more glandular where they have more exposure to water.

Lineation

LINEATION increases with WARMTH and LATITUDE about equally, which is a reflection of the geographic pattern of low values in the

east both in the north and south (Figure 25, Pattern 3). The path diagram variables (Figure 33) explain more of the variation relative to a TSA than they do for SPOTTING and GLANDS (a little less than the fourth TS step for a TSA of LINEATION-CC3), so the locality variables are more important than the geographic pattern. The opposite signs of the coefficients of variables that describe wetland and water in the habitat suggest strong environmental influences on LINEATION. WATER AREA within 5 km decreases LINEATION, but LAKE SIZE and RIVER WIDTH increase it and WETLAND SIZE and SWAMP AREA decrease LINEATION while MARSH AREA increases it, so LINEATION increases in proximity to large bodies of water and in wetland that is marsh rather than swamp.

Several workers (Pyburn, 1961; Fishbeck & Underhill, 1971; Nevo, 1973a; Stewart, 1974; Schueler & Cook, 1980) have described situations in which the frequency of dorsal striping is higher in populations of frogs in grassier or otherwise linearly patterned habitats, and this explanation seems to account for the variation in LINEATION seen in the present data.

Specimen Size

Schuster (1950) "discovered few patterns in the geographic variation in morphology in European amphibians [but] . . . he did conclude that among amphibians in the warmest part of their ranges, drought-resistant forms were largest while humidity-dependent species were smallest" (Jameson *et al.*, 1973 p. 286). Among North American hylids, *Acris* (Nevo, 1973b) are larger in drier climates, while *Hyla regilla* (Jameson *et al.*, 1973) are smaller there. Among the skins *Rana pipiens* seems to fit the drought-tolerant model. SKIN SIZE decreases with increased MARSH AREA and SWAMP AREA, and is most highly correlated with ARIDITY, though its coefficient in the final regression is relatively low. As in the case of LINEATION (both are most similar to Geographic Pattern 3) the inverted coefficients of WARMTH and LATITUDE (Figure 34) probably reflect the smaller body size of the northern and southern eastern samples. The adaptive sense of this is that larger frogs, with a smaller surface-volume ratio, take longer to dehydrate in dry situations. This pattern should not be taken too seriously until it is confirmed by description of size variation among breeding adults.

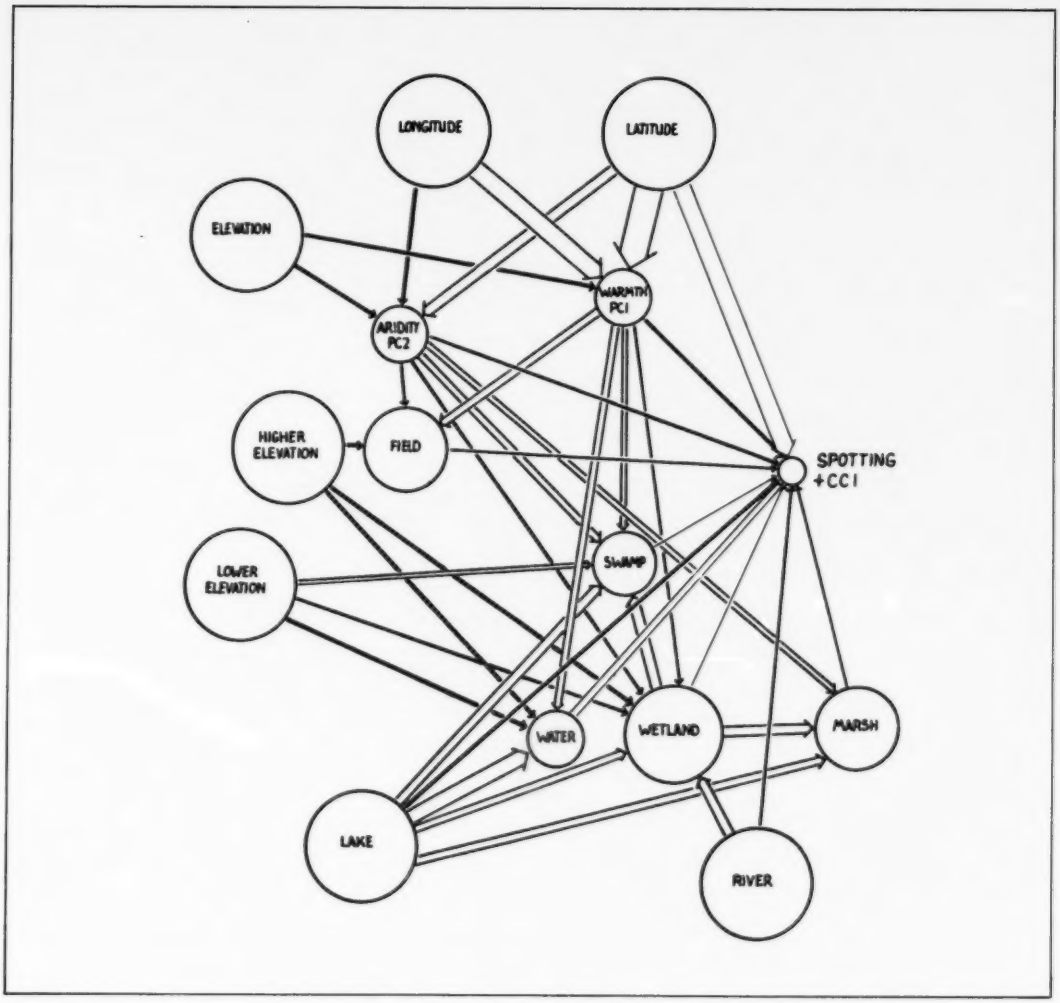

Figure 28. Path diagram of SPOTTING+CC1.

The path coefficients leading to SPOTTING+CC1, in the order of stepwise inclusion in the regression equation, and variance explained at each step are:

LATITUDE	-.82	63%	LAKE SIZE	0.17	76
WARMTH	-.22	71	MARSH AREA	-.10	76
WATER AREA	-.33	75	FIELD AREA	-.11	77
ARIDITY	-.15	76	WETLAND SIZE	-.05	77
RIVER WIDTH	0.14	76	SWAMP AREA	-.04	77

(about 84% of the variation is among localities)

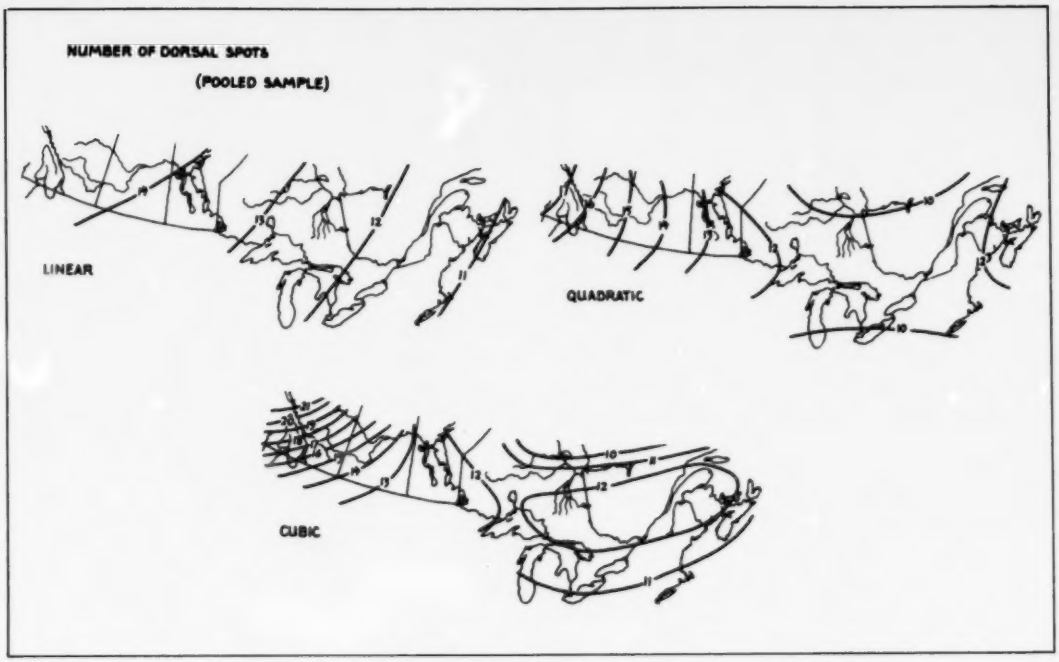

Figure 29. Geographic variation in NUMBER OF DORSAL SPOTS: TSA of pooled samples.
This analysis is based on the variation among the pooled skin and fluid-preserved specimens. The coefficients are:

	LINEAR	QUADRATIC	CUBIC
Constant	13.87915	13.03458	14.75007
E	-.06074081	-.1937506	-.4219567
N	0.09329507	0.7421236	-.6171147
E**2		0.003106076	0.01399859
E*N		-.008727875	0.003459746
N**2		-.02926204	0.1683296
E**3			-.0001536557
(E**2)*N			0.0003782645
(N**2)*E			-.0027384
N**3			-.005504043

E=120-Longitude, N=Latitude-40

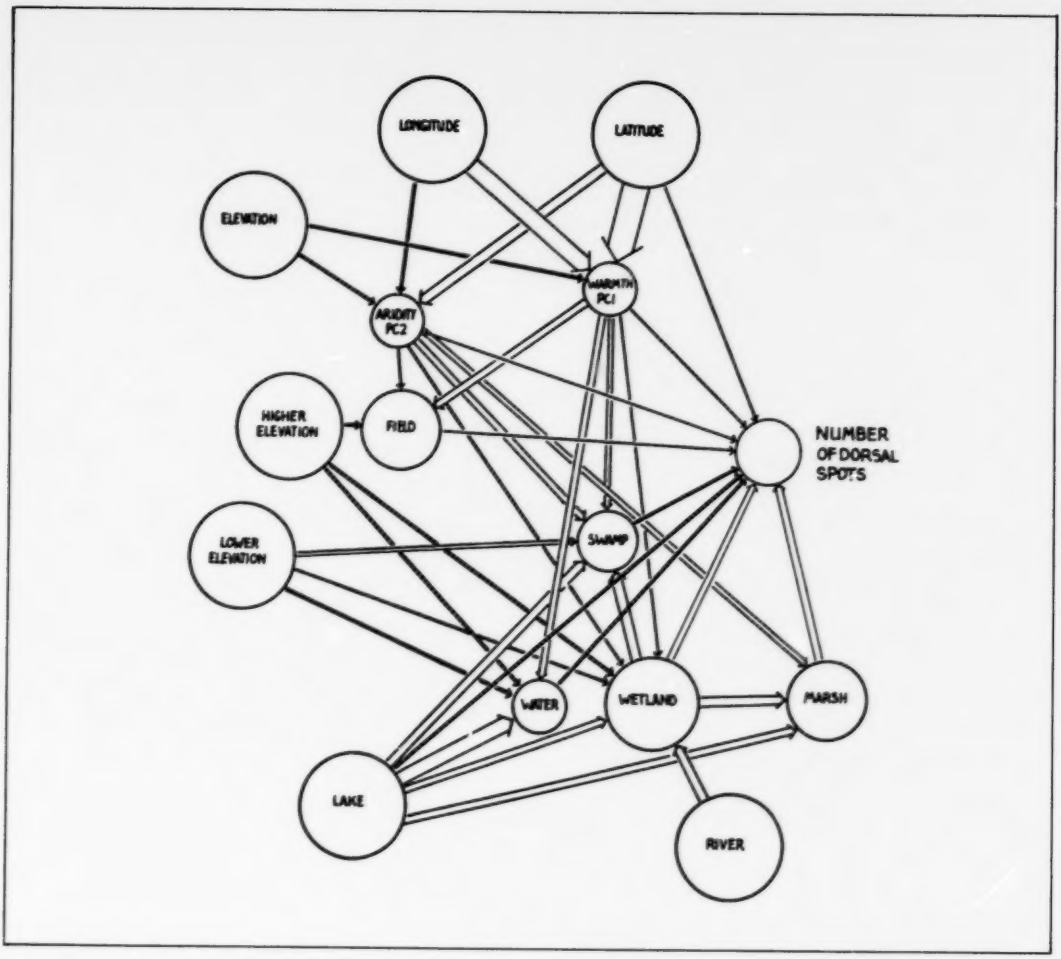

Figure 30. Path diagram of NUMBER OF DORSAL SPOTS.

The path coefficients leading to DORSAL SPOTS, in the order of stepwise inclusion in the regression equation, and variance explained at each step are:

LATITUDE	0.16	5%	SWAMP AREA	0.24	11
WATER AREA	-.22	8	FIELD AREA	0.10	11
LAKE SIZE	0.17	8	WARMTH	-.16	11
WETLAND SIZE	-.30	9	ARIDITY	0.10	12
MARSH AREA	0.27	10			

(about 20% of the variation is among localities)

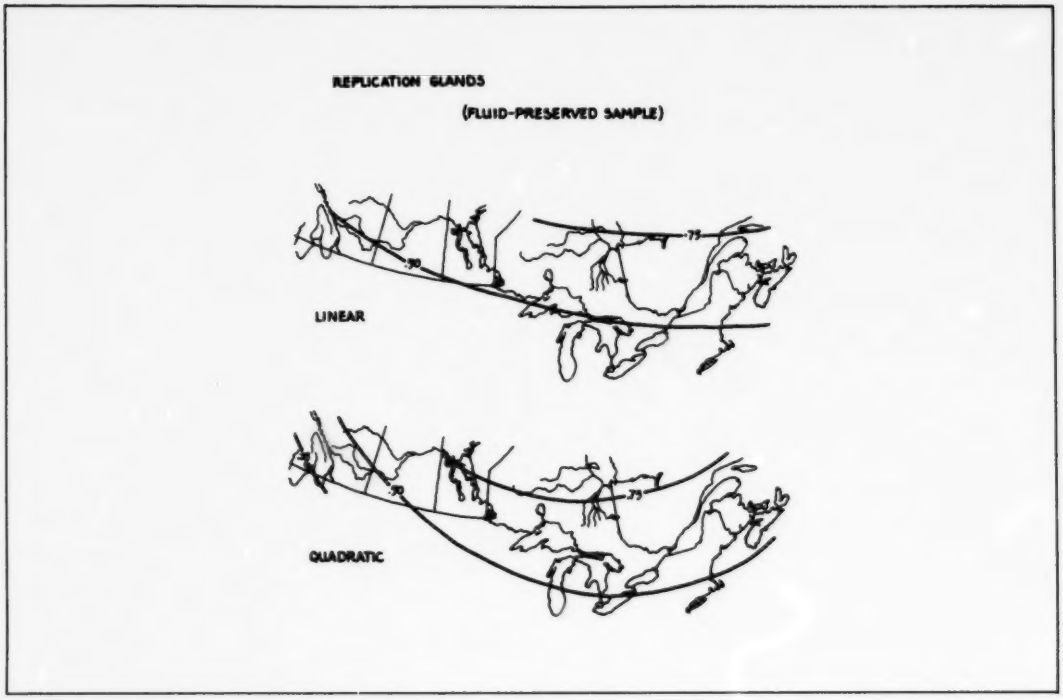

Figure 31. Geographic variations in GLANDS: TSA of fluid-preserved sample.
This analysis is based on the REPLICATION GLANDS variable among only the fluid-preserved specimens, since although these surfaces are highly correlated with the variation among the skins (Table 17) the values of REPLICATION GLANDS in the two samples differ, and the fluid-preserved sample covers a greater geographic area. The scale is standard deviations from the mean of the skin sample. The coefficients are:

	LINEAR	QUADRATIC
Constant	-.09220853	0.2090565
E	-.006291279	-.02797246
N	-.03313606	-.03373365
E**2		0.0003029435
E*N		0.0001797839
N**2		-.0006778907

E=120-Longitude, N=Latitude-40

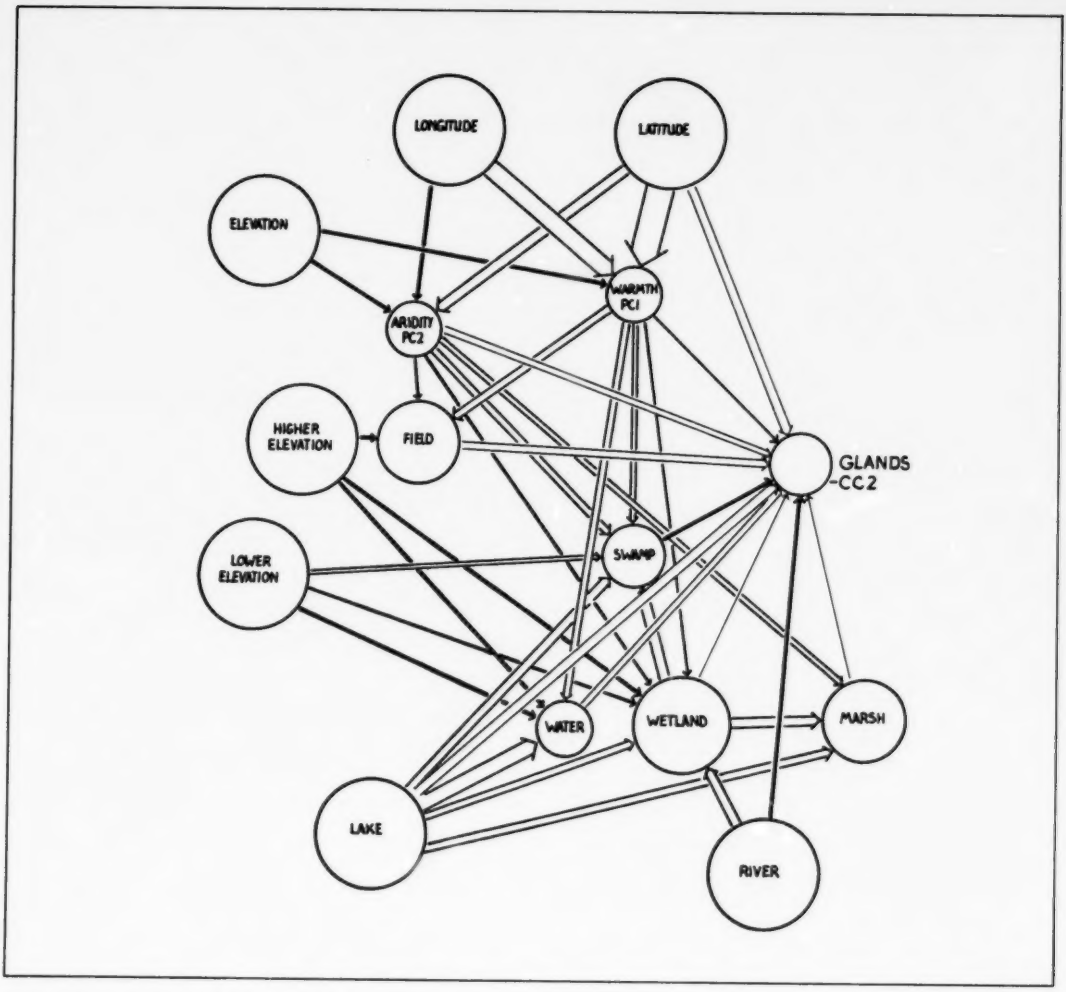

Figure 32. Path diagram of GLANDS-CC2.

The path coefficients leading to GLANDS-CC2, in the order of stepwise inclusion in the regression equation, and variation explained at each step are:

WETLAND SIZE	-.03	19%
WARMTH	0.08	33
ARIDITY	0.36	37
FIELD AREA	0.33	39
LAKE SIZE	-.46	40
WATER AREA	0.27	41
RIVER WIDTH	-.20	42
SWAMP AREA	-.18	43
LATITUDE	-.45	45
MARSH AREA	0.03	45

(about 68% of the variation is among localities)

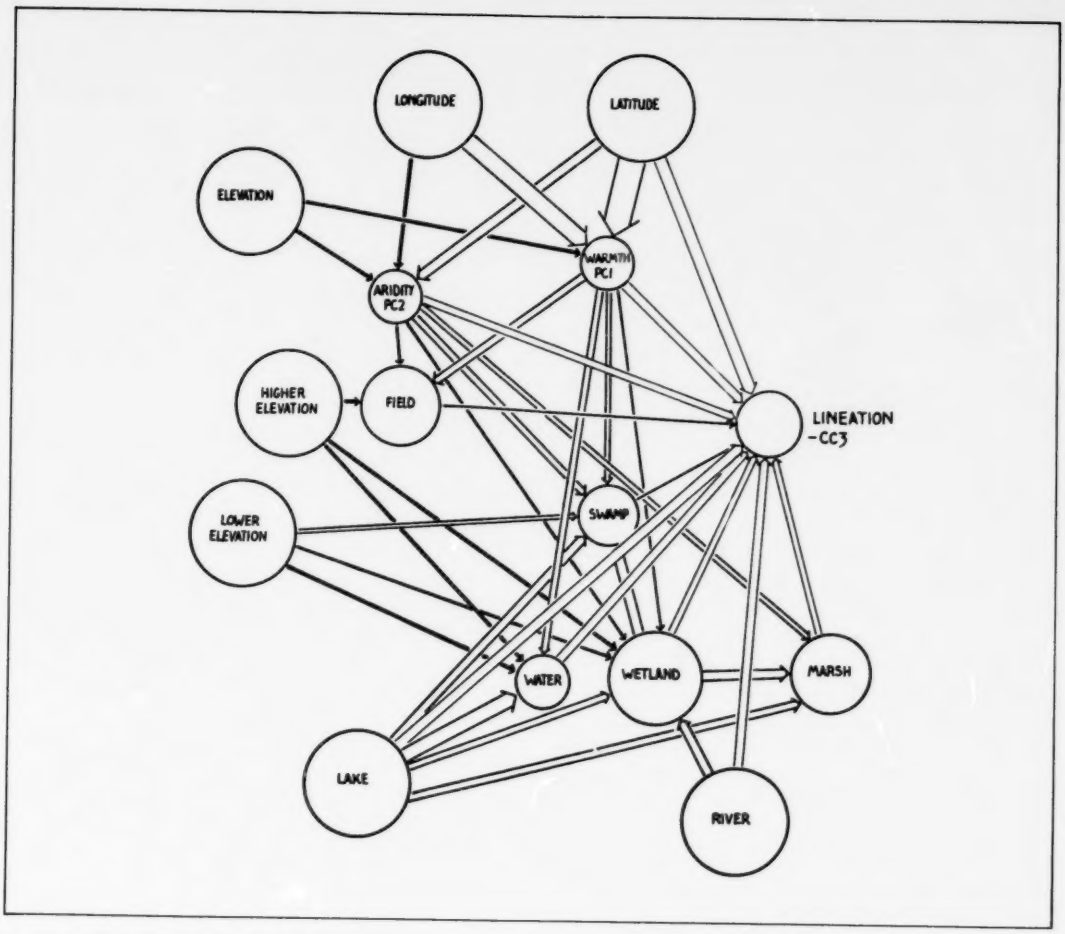

Figure 33. Path diagram of LINEATION-CC3.

The path coefficients leading to LINEATION-CC3, in the order of stepwise inclusion in the regression equation, and variation explained at each step are:

MARSH AREA	-.27	7%	WATER AREA	0.42	18
FIELD AREA	0.12	13	LAKE SIZE	-.45	21
SWAMP AREA	0.11	14	WETLAND SIZE	0.36	23
RIVER WIDTH	-.38	16	LATITUDE	-.58	24
WARMTH	-.50	17	ARIDITY	0.34	25

(about 45% of the variation is among localities)

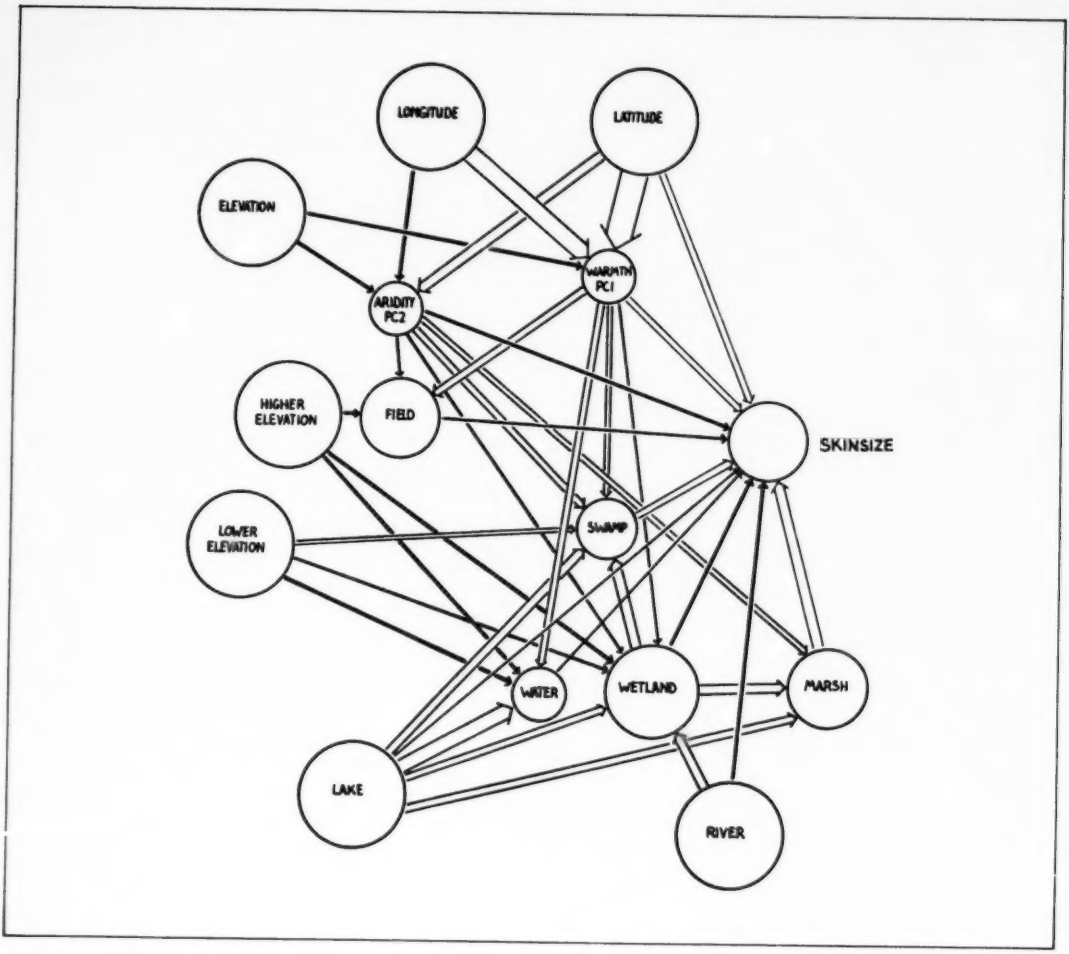

Figure 34. Path diagram of SKIN SIZE.

The path coefficients leading to SKIN SIZE, in the order of stepwise inclusion in the regression equation, and variance explained at each step are:

ARIDITY	0.19	13%	WETLAND SIZE	0.18	19
RIVER WIDTH	0.21	14	WARMTH	0.32	19
MARSH AREA	-.40	16	LATITUDE	0.35	20
LAKE SIZE	0.11	17	FIELD AREA	-14	20
SWAMP AREA	-.28	18	WATER AREA	-.09	21

(about 46% of the variation is among localities)

Part VI: Conclusions

Biological Conclusions

The data of geographic variation are remote from causal interpretation and can at best give only moderately compelling support to any adaptive hypothesis; I believe that the following generalizations have received such support in the present study:

1) The strongest pattern of pigmentation variation in *Rana pipiens* is the greater extent and connectedness of dark pigmentation in warmer, moister climates: or geographically, in the vicinity of the Great Lakes drainage. This variation conforms to the pattern called Gloger's Rule, and may facilitate visual crypsis by matching locally prevalent backgrounds. This does not seem to be a general rule in *Rana*, and may, in part, be a result of a more aquatic niche of populations of *R. pipiens* allopatric from aquatic species of *Rana* (which characteristically have little dark pigmentation).

2) Variation in the extent of dermal secretory glands is largely independent of variation in pigmentation: the geographic pattern is one of more extensive glands in the boreal forest than in the prairies or Great Lakes drainage. Superimposed on this is a tendency for more extensive glands in habitats where water is more widespread, which suggests that the glands serve to protect the frogs from some stress associated with water (perhaps infection or osmotic losses).

The occurrence of more extensive dermal glands in more aquatic habitats may be a general rule among semi-terrestrial *Rana*.

3) A third independent kind of variation is an association between field, lake edge, and marsh habitats and linear pattern elements and reduced spotting on the hind legs; this is likely cryptic against the grassier backgrounds on which frogs in such habitats must be viewed by predators.

4) These three patterns emerge in factor analysis of multistate data, DFA among localities, and CCAs with environmental and geographic variables. The first two retain their independence when replicated on data from another set of specimens, so they seem to be fairly robust aspects of variation among populations of *R. pipiens*.

5) East of the Cordillera, at least, much of the variation in the number of spots is within rather than among populations. The among-populations variation in dorsal spot number is inverse to that in spot area, so the variation is compatible with an hypothesis of disruptive coloration maintained by apostatic selection. There is a step-like increase in the number of dorsal spots near the northwestern limits of the species range in British Columbia.

6) Insofar as this can be evaluated on counts, multistate characters, and measurements of nonstructural features, within- and among-population variation is positively correlated.

7) The first two patterns of variation within local populations, once size is accounted for, are similar to the first two patterns of variation among populations: variation in the extent of spotting and glands.

8) There is no evidence that there is covariation among the attributes examined in within-population variability, as in a PCA of the standard deviations of variables each of the first 7 PCs is dominated by the variation of one locality or one attribute.

9) The sexes of *R. pipiens* differ significantly, but not markedly, in pigmentation, though not in dermal glands, or dorsal spot number. Males are less patterned on the head and back than females are, but they are more patterned on the lateral and ventral surfaces of the body. These differences may reflect selection for males coloured more like aquatic frogs, since they spend more of the spring breeding period in aquatic habitat than females do.

10) There is ambiguous evidence from several analyses which suggests that female pigmentation is more sensitive to environmental variation than that of the males, as if females are exposed to more visual predation over more locally variable backgrounds (*i.e.*, terrestrial summer habitat) than are males (*i.e.* spring breeding ponds).

11) *R. pipiens* which are phenotypically much like the burnsi and kandiyohi morphs occur outside the midwest of the United States: apparent burnsi (dorsally unspotted frogs) at several localities, and apparent kandiyohi at Long Point, Ontario. Frogs much like the 'pseudokandiyohi' of Merrell (1965) occur in the prairies of southern Manitoba and Saskat-

chewan and North Dakota, but not elsewhere in the sample studied here.

Methodological Conclusions

I have assumed that "in order to ascertain the patterns of genetic and morphological variation which exist in nature, and to seek to explain those patterns with nontrivial, predictive, and falsifiable hypotheses, it is necessary to adopt a quantitative, multivariate methodology that concentrates on the evolution of populations" (Gates, 1978, p. 221) and have not explicitly evaluated any of the techniques I have used in this study. My conclusion that this methodology is worthwhile depends upon this assumption, the translation of evolutionary hypotheses into the ritual language of multivariate analysis, and my satisfaction with the results. I commend the following translations to other students of geographic variation:

1) The use of trend surface regression analysis as a 'null hypothesis' of difference associated with distance to compare to hypothesized environmental or taxonomic relationships.

2) The use of canonical correlations analysis between specimen and geographic variables (terms of TSA) to extract uncorrelated kinds of geographic variation from the specimen data.

3) The comparison of patterns of variation in *a priori* composite variables (indices, estimates of parameters, discriminations between similar species) as an aid to the interpretation of statistically generated *a posteriori* variables.

4) The use of habitat and relief descriptors derived from topographic maps in the search for local influences on among-sites variation.

5) The treatment of climatic data not gathered at the site of collection of the specimens as geographic patterns described by high-order trend surfaces.

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The Herpetology and Ichthyology sections of the NMC have unstintingly provided me with working space, encouragement, and field supplies; they have catalogued a diversity of unconventional specimens, and have been more than generous in allowing me to use the herpetological collection and the ichthyological computing facilities. Mike Rankin provided a vehicle at a time when transportation was critical.

Aleta Karstad caught frogs, prepared skins, wrote labels, recorded data, drew the figures, typed the first draft of the manuscript and much of the second, checked the growth of unnecessary hypotheses, and cheerfully endured several years of indenture to two sysiphean manuscripts (see also Karstad, 1979).

W.L. Brown first directed my interests toward the systematics of leopard frogs. J.D. Rising suggested that I make this the subject of my thesis (Schueler, 1979), and patiently awaited its completion through many years.

My analytic philosophy is indebted to two other doctoral theses in the Zoology Department of the University of Toronto: to Jake Rice's study of *Vireo* ecology (1974, 1978a-c) for the lesson that multivariate analysis can be an expression of an hypothesis rather than just an oligovariate description of variation, and to Michael Gates' study of *Euplotes* systematics (1976, 1977) for the idea of using variation accounted for by one class of independent variables as a control for variation accounted for by putatively causal variables. These lessons are available elsewhere, but I did not learn them there.

Early drafts of the manuscript were carefully read by Francis Cook, Don McAllister, Michael Gates, Jim Rising, Aleta Karstad, E.J. Crossman, and G.K. Morris. I have also had useful discussions of the matter treated here with R.O. Brinkhurst, Ralph Gibson, John Foster, Grant Sheng, Søren Bondrup-Neilsen, Frank Ross, Thomas Parsons, Roger Hansell, Garry Sprules, Allan Baker, and Rick Elinson. I remained at the University of Toronto long enough to complete the thesis only because Sheila Freeman successfully buffered my interactions with the School of Graduate Studies.

I list all of this assistance and thank everyone who has given it, but I am in no sense apportioning responsibility for errors by doing this. Errors and omissions are largely due to my failure to obtain a mathematical understanding of the statistical procedures I have used, my clumsiness at experimentation, my ignorance of histological methods, and my disorderly habits of analysis and composition. There are, of course, too few specimens from too few localities: I can only plead that I went to many other places in pursuit of leopard frogs without obtaining any.

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Appendix 1: Skin Characters

Pigmentation Definitions:

Spot: An area of dark pigment with a sharp boundary between it and the adjacent less pigmented area.

Smudge: An area of dark pigment without a sharp boundary between it and the adjacent less pigmented area.

Primordium: A continuous area of dark pigment without any constrictions of the margin indicating a fusion of more than one such area; the portion of any spot or smudge between or beyond such constrictions (='spot' of Merrell, 1965; the anteriormost lefthand dorsal spot in Figure 4 comprises two primordia).

Bar: A spot on the dorsal surface of the hind leg, generally with its long axis perpendicular to that of the leg.

Full bar: A bar which extends perpendicularly at least most of the way across the dorsal surface of the leg.

Diagonal bar: A full bar which crosses the midline of the dorsal surface of the leg at less than about 60° (generally near the knee).

Half bar: A bar which does not extend across the midline of the dorsal surface of the leg.

Broken bar: Two half bars more or less adjacent across the midline of the dorsal surface of the leg.

Multistate Characters

Nose Spots: The number of spots between the canthus-rostralis lines.

Left Eyelid Spots, Right Eyelid Spots: The number of spots on the upper eyelids.

Nostril Spot: The intensity of pigmentation around the nostrils:

0) absent
1) a smudge surrounding or bordering on the nostril
2) a spot surrounding or bordering on the nostril

Canthus-Rostralis Line: The extent and intensity of pigmentation along the line between the anterior canthus and the tip of the snout (excluding the nostril spots):

0) absent
1) a discontinuous spot or smudge
2) a continuous smudge lighter than the back spots
3) a continuous spot or smudge as dark as the back spots

Tympanum Spot: The presence and colour of a spot at the centre of the tympanum, the extreme value between the sides:

0) a dark spot
1) absent
2) a light spot

Tympanum Outlining: The number of quadrants of the tympanum surrounded by spot or smudge pigmentation, averaged between sides and rounded down to an integer.

Jaw Mottling: The extent of dark or dusky spots on lower jaw:

0) less than 3 primordia
1) 3 to 10 primordia
2) more than 10 primordia

Number of Dorsal Spots: The number of spots between the dorsolateral folds posterior to the eyelids.

Number of Merged Dorsal Spot Primordia: The number of spot primordia merged with other primordia, between the dorsolateral folds and posterior to the eyelids

Spot Outlining: The presence of a light ring around the dorsal spots:

0) absent
1) pale, grading gradually outward from the spot edge
2) much paler than back and more or less distinctly bounded distally

Dorsolateral Fold Coloration:

0) concolour with back
1) paler than back
2) much paler than back, distinctly bounded

Dorsal Spot Bilinearity: The extent to which the dorsal spots are arrayed in 2 anterior-posterior rows:

0) no rows
1) spots in 3 rows

2) spots in 2 rows with 1 or 2 spots out of line
3) spots in 2 rows with no spots out of line

Lateral Spot Reticulation: The degree of spot or smudge primordium fusion on the flank:
0) no fusion of 3 or more primordia (except poorly defined smudge primordia in the groin)
1) fusion of 3 or more primordia into one pigmentation
2) half or more of the pigmented flank covered by such pigmentations
3) the entire pigmented flank reticulated

Lateral Spot Lineation: The number of clear rows of spots per side, averaged between sides and rounded down to an integer.

Femur Background Colour: The background colour of the posterior face of the thigh (see Moore, 1944):
0) light
1) indeterminate
2) dark

Femur Patterning: The pattern of pigmentation on the posterior face of the thigh (see Moore, 1944):
0) spots
1) intermediate
2) reticulation

Femur full bars
Femur half bars
Femur broken bars
Femur diagonal bars
Tibia full bars
Tibia half bars
Tibia broken bars

These are counts of the number of spots of each spot type on the dorsal (pigmented) faces of both thighs and one calf.

Jaw dermal glands
Throat dermal glands
Tibia dermal glands

These characters describe the extent of ventral glandulation, as defined in Figure 5.

Female Paleness: Intensity of dark pigmentation on the posterior face of the thigh:
0) as dark as dorsal spotting
1) much paler than dorsal spotting
2) barely distinguishable from background colour

Duskiness: The presence of visible melanophores over most of the ventral surface:
0) absent
1) present

Extent of Dark Jawline: The extent of dark pigmentation along the upper jaw:
0) absent
1) one to four isolated spots per side, no spots more than four times as long as wide
2) spots extending along less than 60% of the length of the jaw
3) spots extending along more than 60% of the jaw
4) a continuous dark line with light flecks
5) a continuous solid dark line with no more than one break

Intensity of jawline: The darkness of the pigmentation of the dark line on the upper jaw:
0) absent
1) much paler than the back spots
2) about the same intensity as the back spots

Extent of light jawline: The extent of a line lighter than the adjacent skin along the upper jaw:
0) absent
1) not extending beyond the anterior canthus
2) not extending beyond the nostrils
3) extending continuously beyond the nostrils on both sides

Metric Characters

Nose spot length: The anterior-posterior length of the longest nose spot.

Skin length: The distance along the median line from the pineal spot to the inguinal septum.

Longest gland line: The length of the longest continuous line of dermal glands between the dorsolateral folds.

Longest spot: The anterior-posterior length of the longest dorsal spot.

Femur line: The length of dark pigmentation distal to the main femur spots along a line from the distal point of the knee to the inguinal septum.

Femur midline: The length of the dorsal midline of the femur from the inguinal septum to the distal point of the knee.

Femur midline spotted: The length of dark pigmentation along the femur midline.

Tibia length: The distance from the knee to the heel along the distal face of the pigmented part of the tibia.

Tibia line: The length of dark pigmentation along the tibia length.

Grid Characters

Dorsal spotted: Dorsal points falling on dark pigmentation.

Dorsal unspotted: Dorsal points not falling on dark pigmentation.

Unspotted-no glands: Tibia points not falling on dark pigmentation or glands.

Unspotted-glands: Tibia points not falling on dark pigmentation, falling on glands.

Spotting-no glands: Tibia points falling on dark pigmentation, not falling on glands.

Spotted-glands: Tibia points falling on glands in dark pigmentation.

Appendix 2: Coefficients for 6th order Climate Trend Surfaces.

X=50-((Longitude-100)*Cosine(Latitude)); Y=Latitude-15; ELEV=Elevation in metres. Two elevation terms entered each regression, stepwise, so the elevation variables in each equation are indicated above the coefficients.

	WARMTH	ARIDITY	SUMMER HUMIDITY	WIND	NO FOG	FOG & SNOW
Constant	-2.275625E+02	-2.299447E+03	8.375072E+02	3.316731E+03	-2.056058E+03	-1.554327E+03
X	1.774818E+01	1.748899E+02	-6.412906E+01	-2.795246E+02	1.766971E+02	1.329198E+02
Y	1.364239E+01	1.65634E+02	-4.41096E+01	-1.930929E+02	1.195854E+02	8.367654E+01
X2	-5.511219E-01	-5.39417	2.088041	9.518797	-6.19271	-4.44645
XY	-9.161485E-01	-1.052047E+01	2.62933	1.346434E+01	-8.564867	-6.59742
Y2	-3.188047E-01	-4.828107	8.861121E-01	4.719353	-2.963246	-1.252013
X3	8.808967E-03	8.680846E-02	-3.609325E-02	-1.644758E-01	1.133334E-01	7.166889E-02
X2Y	2.434305E-02	2.602323E-01	-6.668047E-02	-3.782968E-01	2.375274E-01	2.052923E-01
Y2X	1.665025E-02	2.466428E-01	-3.703958E-02	-2.454573E-01	1.752728E-01	8.398916E-02
Y3	3.64053E-03	7.060716E-02	-7.321302E-03	-6.777919E-02	3.536911E-02	1.455229E-03
X4	-7.774605E-05	-7.857388E-04	3.329888E-04	1.473713E-03	-1.12238E-03	-5.517791E-04
X3Y	-3.196164E-04	-3.111982E-03	9.247E-04	5.304624E-03	-3.339308E-03	-3.000564E-03
X2Y2	-3.167786E-04	-4.574234E-03	5.662691E-04	4.97406E-03	-3.369737E-03	-2.391648E-03
Y3X	-1.563107E-04	-2.834251E-03	1.950176E-04	2.301355E-03	-2.034747E-03	4.465794E-05
Y4	-1.025866E-05	-4.90403E-04	1.506959E-06	6.576864E-04	-5.687758E-05	7.730108E-05
X5	3.903776E-07	4.028807E-06	-1.394096E-06	-6.08282E-06	5.389787E-06	1.719042E-06
X4Y	1.956472E-06	1.74342E-05	-7.095931E-06	-3.686608E-05	2.496939E-05	1.913486E-05
X3Y2	2.982427E-06	3.756651E-05	-3.556506E-06	-4.496473E-05	2.554093E-05	3.100088E-05
Y3X2	1.474845E-06	3.475733E-05	2.425032E-06	-3.042515E-05	2.94982E-05	7.115419E-07
Y4X	8.658128E-07	1.527563E-05	1.608659E-06	-1.023956E-05	9.329523E-06	-4.050431E-06
Y5	-1.800941E-07	1.160321E-06	-1.922782E-07	-4.713385E-06	-2.053081E-06	-4.904562E-07
X6	-1.064432E-09	-1.02269E-08	1.357087E-09	7.466979E-09	-8.212323E-09	-1.62538E-09
X5Y	-3.472753E-09	-3.220423E-08	2.280802E-08	9.931058E-08	-8.028219E-08	-3.364483E-08
X4Y2	-1.382602E-08	-1.23948E-07	1.174935E-08	1.580641E-07	-7.29419E-08	-1.545664E-07
X3Y3	3.992606E-10	-1.203336E-07	-2.034209E-09	1.223929E-07	-9.730073E-08	1.183254E-09
Y4X2	-9.612189E-09	-1.195547E-07	9.285302E-09	8.317876E-08	-1.201991E-07	3.502913E-09
Y5X	2.005036E-09	-1.509277E-08	-2.364768E-08	5.169694E-09	1.52345E-08	3.109008E-08
Y6	2.694194E-10	-2.375252E-09	6.777594E-09	2.149128E-08	6.146202E-09	-3.31433E-09
	ELEV	ELEV	ELEV	ELEV**3	ELEV**3	ELEV
	-2.950674E-04	1.738254E-03	-6.783027E-04	-4.338128E-10	5.992975E-10	1.950296E-03
	ELEV**2	ELEV**2	ELEV**2	ELEV**4	ELEV**4	ELEV**2
	-1.760808E-07	-5.725792E-07	2.925961E-07	1.896473E-13	-2.962481E-13	-5.433375E-07

Appendix 3: Coefficients for Geographic Patterns

Variable	Linear Pattern 1	Quadratic Pattern 1	Cubic Pattern 1	Linear Pattern 2
Constant	-4.68356E-02	7.17015E+00	-1.23454E+01	-7.10347E+00
E	3.62878E-02	-2.45353E-01	2.00281E-01	1.20525E-01
N	-2.12587E-01	-7.32094E-01	4.57463E+00	4.65993E-01
E**2	0.0	2.19029E-03	5.46795E-03	0.0
E*N	0.0	1.81933E-02	-1.00017E-01	0.0
N**2	0.0	-1.25260E-02	-4.17481E-01	0.0
E**3	0.0	0.0	-8.90413E-05	0.0
(E**2)*N	0.0	0.0	3.38950E-04	0.0
(N**2)*E	0.0	0.0	5.91298E-03	0.0
N**3	0.0	0.0	7.74331E-03	0.0

Variable	Quadratic Pattern 2	Cubic Pattern 2	Quadratic Pattern 3	Cubic Pattern 3
Constant	-5.49557E-01	6.00175E+01	-2.24577E+01	4.96511E+01
E	-1.38915E-02	-2.90083E+00	3.48955E-01	-3.77581E+00
N	-2.60979E-01	-1.84443E+01	3.63735E+00	-1.06162E+01
E**2	-4.17692E-04	5.45970E-02	1.71330E-03	8.42273E-02
E*N	1.64712E-02	4.93037E-01	-4.70649E-02	4.58714E-01
N**2	2.80590E-03	1.51230E+00	-1.21990E-01	7.09044E-01
E**3	0.0	-4.37336E-04	0.0	-5.79671E-04
(E**2)*N	0.0	-2.39233E-03	0.0	-4.38936E-03
(N**2)*E	0.0	-2.53891E-02	0.0	-1.65964E-02
N**3	0.0	-2.51385E-02	0.0	-8.83510E-03

E=120-Longitude, N=Latitude-40